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## NOTICE TO SUBSCRIBERS OF GENETICS

The exigencies of war necessitate two changes in GENETICS which become effective with this issue. GENETICS was formerly printed on 11-point type. It will now be on 10-point. In this way we can print more words per page without loss of legibility and thus conserve a decreasing supply of paper. In addition, printing costs will be reduced slightly. This change in size of type will result in fewer printed pages and hence thinner issues.

The second change is the temporary elimination of the frontispiece for the duration of the war or until the financial condition of the journal improves. Beginning with the first volume of GENETICS, the portrait of a distinguished geneticist with an accompanying biographical sketch appeared each year as the frontispiece of the January issue. This has been an attractive feature of the journal. It is, however, of considerable expense. In view of the loss of all foreign subscriptions, the Editorial Board decided to devote our dwindling resources to the publication of experimental results. It is to be hoped that the frontispiece can be restored in the near future.

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# CHROMOSOME STRUCTURE IN THE ACRIDIDAE WITH SPECIAL REFERENCE TO THE X CHROMOSOME

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EXCEPT in the case of forms in which chromosomes with a median or submedian constriction have been found, the somatic metaphase chromosomes in both the Acrididae and the Tettigonidae (grasshoppers) have commonly been described and illustrated as longer or shorter rods with a terminal spindle attachment region. Such chromosomes have been designated as telomitic or telocentric as opposed to the atelomitic or mediocentric chromosome forms which have been generally found both in the animal and the plant kingdom. It is true that more than one investigator has expressed doubts as to the existence of really telomitic chromosomes as stable and persistent forms. Thus HEITZ (1935) holds that all supposedly telomitic chromosomes possess a short second arm which, either because of its minute size or because of faulty fixation, is not visible in microscopic preparations (see also RHOADES 1940).

This view has apparently not been generally accepted by cytologists who have investigated the chromosomes of the Acrididae, since most workers describe and illustrate these so-called "telomitic" or "telocentric" chromosomes as having strictly one arm and a terminal spindle fibre attachment. WHITE (1941), who is more cautious than most, expresses his views as follows: "The term telocentric will be used throughout this paper to indicate a chromosome in which the centromere is effectively terminal, so that the chromosome consists of one limb only (if a second limb is present, it is so minute that, for most purposes, it can be disregarded)." His illustrations of early spermatogonial metaphases of *Schistocerca gregaria* Forsk., *Melanoplus femur-rubrum* DeGeer, and *Chorthippus viridifasciata* DeGeer give no evidence of a second arm, and as already stated, this applies also to most other figures of grasshopper metaphase chromosomes that have come to the writer's attention. DARLINGTON (1936) appears to be the only investigator who has shown clearly the presence of a second arm in the rod-shaped chromosomes of Acrididae. In his figures and descriptions of the metaphase chromosomes of *Chorthippus parallelus* he shows clearly the presence of a distinct short arm on the rod chromosomes so that there are in this form five somatic pairs plus the X chromosome showing subterminal constrictions along with the three pairs of long chromosomes with submedian constrictions.

The accompanying photomicrograph (fig. A) shows the last spermatogonial metaphase of *Chortophaga viridifasciata*. This is from a testis squash preparation pretreated for ten minutes in Ringer's solution containing NaCN $2^{-3}$ mol, fixed in acetic alcohol (1:2), Feulgen stained, squashed on the slide, and mounted in dioxane balsam. In the photomicrograph, all the 23 chromosomes of the diploid complement can be seen and, in the case of most (14 out of the 23), there is evident a very distinct short arm separated from the long arm by an unstained primary constriction (spindle attachment). A careful examina-

tion of this and similar preparations makes it quite clear that a distinct short arm is present in all the chromosomes of the complement including the very short ones and also the X chromosome. These do not all show clearly in the illustration, but enough are visible to indicate that a short arm is characteristic and that telomitic chromosomes do not exist in this form.

In addition to the presence of short arms, the illustration also shows that the size of the short arm varies considerably throughout the complement. The long chromosome marked "a" has a very small short arm which, however, is distinct enough to show the chromatid split. The medium lengthed chromosome marked "b" has a much larger short arm. Similarly the short chromosomes show a marked difference in the length of the short arm. The one marked "c" has a very small short arm, while the two marked "d" have a short arm about one-third the length of the long one. No attempt has been made to classify the chromosomes of the complement on the basis of the length of the short arm, but undoubtedly it will serve, along with the total length, as a valuable criterion in this connection.

The atelomitic structure of chromosomes is not confined to the genus Chortophaga. A similar structure has been established in the case of a number of other members of the Acrididae, notably in species of the genus Melanoplus. The writer is of the opinion that the phenomenon is general in the Acrididae as a whole, and it is probable that so-called telomitic chromosomes in other Orthopteran groups have a similar structure and that drawings showing this condition are, in general, incorrect. It is evident that this has resulted from faulty fixation leading to a shrinkage which has obscured the primary constriction. A contributory factor may have been the usual method of staining (iron haematoxylin), with which it is much more difficult to differentiate a primary constriction than with a very selective microchemical stain such as provided by the Feulgen reaction. A similar faulty fixation has obscured the real structure of the X chromosome in the spermatocytes of Acrididae.

The spiral structure of somatic metaphase and anaphase chromosomes in plants has been established beyond a doubt. It consists of a simple, rather tightly compressed spiral. The interlocking double spiral structure so frequently described and figured is due to the presence of vacuolation artifacts (see COLEMAN 1940). A similar structure is probably generally present in animal chromosomes but it has apparently not yet been demonstrated. Figure B is a photomicrograph of the last spermatogonial anaphase in *Chortophaga viridifasciata* from material pretreated and stained as described. Since the two anaphases have been squashed more or less on top of one another, it is only in a few places that individual chromosomes stand out distinctly. In some cases, notably in the chromosome marked above with an arrow, the formation of vacuolation artifact is visible. At the point below marked with an arrow a chromosome is shown in which the spiral structure has been retained undamaged. As will be seen, it is a single spiral in all respects similar to that already found in plants.

The X chromosome in the early stages of first meiotic prophase of grasshopper spermatocytes has been generally described as a strongly heteropyc-

notic body usually of a somewhat elongate pear shape and very distinct from the much extended autosomes. This difference applies more especially to the stages commonly designated as leptotene and pachytene and is found both in the Tettigonidae and the Acrididae. However, evidence that the X chromosome as well as the autosomes in the Tettigonidae possesses a spiral structure has existed since 1912, when WILSON (1912) illustrated, in his figures 93–96, a spiral structure in spermatogonial prophases of *Phrynotettix magnus*. In these cases what are illustrated appear to be relic coils of the previous division. MOHR (1916a) found much the same structure in spermatogonial divisions of *Tettigonia* (*Locusta*) *viridissima*. He also pictures, in his figures 40 and 41, the formation of a definite spiral structure of the X chromosome in the late telophases of secondary spermatogonia. He was able to establish in this form that the heteropycnotic X chromosome in the early meiotic prophase really consists of a rather irregularly twisted chromonema, the twists in some cases taking on the form of an irregular spiral (see his figures 70–76). The chromonema of the X chromosome at this stage, as illustrated by him, is very thick, many times the thickness of the synapsed autosomes. It may be noted that he used iron-haematoxylin as a stain after fixation with Flemming-Benda. Later, DE WINIWARTER (1931) found in the spermatogonial prophase and the early meiotic prophase of *Tettigonia albifrons* a structure of the X chromosome very similar to that described and illustrated by MOHR. Finally, WHITE (1940) was able to establish the presence of a spiral structure in both autosomes and X chromosomes in the spermatogonial prophases of seven members of the Tettigonidae and three members of the Acrididae. His paper is illustrated by beautiful photomicrographs of the prophases of various Tettigonidae which bring out the sprial structure of the X chromosome with great clarity. However, WHITE apparently was unable to reveal any structure in the early meiotic prophases of any form. The existence of a structure in the meiotic prophase X chromosome of spermatocytes has not been established in the Acrididae, though the presence of such a structure has been rendered highly probable.

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EXPLANATION OF PLATE

All photomicrographs are from Feulgen squash preparations and are magnified approximately 3000 diameters.

Figure A. Metaphase of last spermatogonial mitosis of *Chortophaga viridifasciata*. The letters a, b, c, d, mark chromosomes showing differences in the relative length of the short arm.

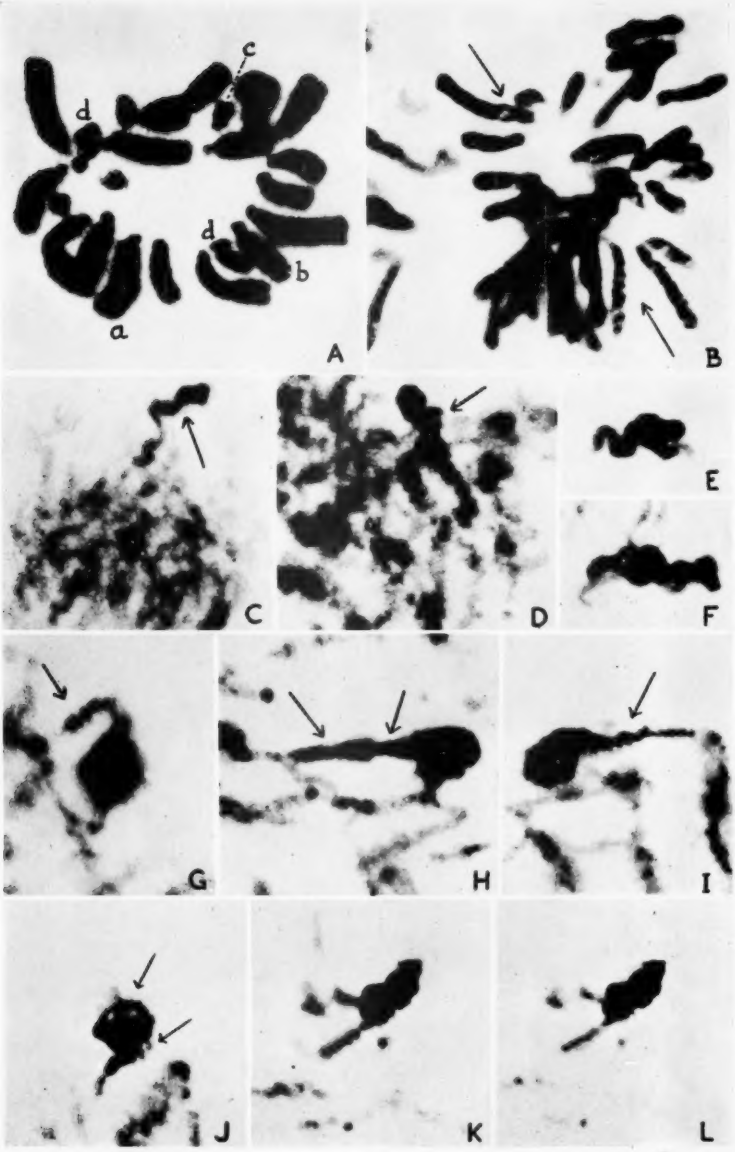
Figure B. Anaphase of last spermatogonial mitosis of *C. viridifasciata*. Above, marked by an arrow, a chromosome shows vacuolation. Below, similarly marked, a chromosome shows the typical single spiral structure.

Figures C and D. Early meiotic prophase (pachytene) in *Melanoplus mexicanus* showing major spiral structure in X chromosome.

Figures E and F. Early meiotic prophase (pachytene) in *C. viridifasciata* showing major spiral structure in X chromosome.

Figures G–J. Early meiotic prophase (pachytene) in *C. viridifasciata* showing minor spiral in drawn out portion of X chromosome.

Figures K and L. Early meiotic prophase in *C. viridifasciata* showing to right and above major spiral and to left and below minor spiral in X chromosome.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The present investigations were confined to two members of the Acrididae, *Melanoplus mexicanus* and *Chortophaga viridifasciata*. The latter of these is identical with one of the three forms studied by WHITE, the former is closely related to one of the other two forms. In both cases testicular tubules were pretreated in Ringer's solution to which a weak concentration of NaCN had been added, fixed in acetic alcohol (1:2), Feulgen stained, and thoroughly squashed on the slide in 50 percent acetic acid. This latter was slowly replaced by dioxane and the squash preparation mounted in dioxane balsam. This method has been found particularly suitable for revealing the finer spiral structure in plant chromosomes (see COLEMAN and HILLARY 1941). In the case of *Melanoplus mexicanus*, the strength of the NaCN was $2^{-5}$ mol and the length of pretreatment five minutes. In the case of *Chortophaga viridifasciata*, the NaCN concentration was increased to $2^{-3}$ mol and the length of pretreatment to ten minutes. The accompanying photomicrographs illustrate the results that were obtained.

Figure C shows a spermatocyte of *Melanoplus mexicanus* in the pachyphase with the X chromosome indicated. Note that to the left the spiral has been drawn out, while to the right it has remained somewhat contracted. The drawn out portion represents the narrow end of the heteropycnotic mass usually figured at this stage. A rather common effect of pretreatment is a loosening or straightening out of the end of the coiled chromonema in this region. In figure D, which also shows a pachyphase of Melanoplus, the X chromonema has been loosened much less, and the gyres of the spiral have remained more or less uniform. In both cases the chromonema is much thicker and more deeply stained than the synapsed autosome pairs at this stage.

The Melanoplus material had a comparatively short pretreatment with a very weak concentration of NaCN. With the stronger and longer pretreatment given the Chortophaga material, more striking and important results were obtained. Figures E and F show results very similar to those obtained in Melanoplus except that the loosened spiral is not so regular. Figure E shows some resemblance to WHITE'S Plate 1, figures 1 and 2. In figure F the gyres are arranged much less regularly, In both cases, the chromonema shows as a uniformly thick thread.

In figure G is represented a condition that was commonly found in Chortophaga material. The main body of the X chromosome has remained unloosened and shows up as a dense uniformly stained mass. One end of the chromonema has loosened and appears much like the loose end of a ball of wool. In this end transverse striations in the chromonema (marked by an arrow in the figure) may be detected, and careful microscopic examination shows that these are due to the existence of a minor spiral. In figures H and I, a much greater length of the chromonema has been unravelled so that the minor spiral is visible over a considerable length of the thread. Arrows indicate where this structure is most clearly revealed. In figure J, the loosened section of the chromonema stands out from the central mass above and to the right, and here the loosening of this portion has been so great as to reveal the minor spiral with great clarity. Finally figures K and L which are prints of different density of the same nega-

tive, confirm the correctness of the interpretation set forth in this paper. To the right the comparatively coarse major spiral is shown. To the left and below the drawn out end of the chromonema, the minor spiral is shown with great clearness. No fewer than five gyres are visible. It might be supposed that these drawn out ends represent, not a terminal portion of the X chromosome, but a precociously condensed autosome pair attached to the X chromosome as has been described and figured by DARLINGTON (1936). This is not the case, since there is no evidence of precocious condensation of autosomes at this stage in either Chortophaga or Melanoplus. There is no doubt that the drawn out portion is in every case an unravelled end of the X chromosome.

It is evident that the structure in the heteropycnotic X chromosome of Acrididae is similar to that revealed in the first meiotic metaphase chromosomes of a number of plant species (see OURA 1936; COLEMAN and HILLARY 1941). The major spiral which appears at first to result from a spiralization of a rather coarse and deeply stained linear thread is really superimposed on a minor spiral, and the thread, in fact, is a very fine one, at least no thicker than the pachytene autosome threads of the same stages. In this thread, however, a chromomeric structure similar to that found in the autosomes has not yet been observed.

WHITE (1940) has already considered the possibility of the presence of a minor spiral to account for the greater thickness of the X chromosome as compared with the autosomes at this stage but has dismissed it with the following words: "there seems no reason to complicate the interpretation of heteropycnosis, by introducing a hypothetical 'minor' spiral into the discussion, particularly since it has so far only been seen in plant chromosomes." He considers the greater thickness of the chromonema to be due to an accumulation of thymonucleic acid, a view which has been very generally accepted to account for heteropycnotic chromosome structure and which has been used recently by DARLINGTON (1942) in support of his theory of "Allocycly." Similar conclusions had been reached by the present writer (COLEMAN 1941) on the basis of earlier observations on Melanoplus X chromosomes but he is now convinced that much, if not all, of the supposed thymonucleic acid accumulation in this particular case is due to the presence of a minor spiral which has heretofore not been seen.

The phenomenon of heteropycnosis in recent years has been intimately associated with the occurrence of what has been looked upon as a special kind of chromatin, heterochromatin, believed to mark chromosome regions which are genically inert (HEITZ 1933). The case has been presented in considerable detail in DARLINGTON'S (1942) recent review so it is unnecessary to go into the matter here. It is sufficient to note that the evidence for the association of heterochromatin with genic inertness is drawn largely from investigations on Drosophila and on the B chromosomes in maize and sorghum (MCCLINTOCK 1933; DARLINGTON and UPCOTT 1941; JANAKI-AMMAL 1940; DARLINGTON and THOMAS 1941). It may be noted in this connection that, as far as concerns the B chromosomes of maize, MCCLINTOCK'S (1933) original photomicrographs show clearly that only a portion of them are heteropycnotic in pachytene.

This point has been emphasized by RANDOLPH (1941) dealing with the genetics of B chromosomes of maize. The evidence advanced by DARLINGTON and THOMAS for heteropycnosis in the case of the B chromosomes of sorghum is anything but extensive.

In the Acrididae, there is no genetic evidence in support of this view. Moreover, in the grasshopper oocyte, the paired X chromosomes show no heteropycnosis (MCNABB 1928; MOHR 1916b), being indistinguishable in this regard from the autosomes, which in some species, but not in *Chortophaga viridifasciata* nor in the forms studied by these authors, show heteropycnotic segments in meiosis. If heteropycnosis is regularly associated with heterochromatin and is characteristic of it, the X chromosomes in the female must be considered as euchromatic. WHITE'S (1940) suggestion that the pairing of X chromosomes in the female is due to the presence of blocks of euchromatin interspersed between the main heterochromatic regions does not explain why there is no evidence of heterochromatin at all in the female. In any case the association of a special heterochromatin with heteropycnosis is entirely lacking in meiosis in the female, and there is as yet no evidence that the X chromosomes in the female differ either in thymonucleic acid concentration or in chiasma frequency from autosomes of similar length. Therefore, there is no justification for associating the heteropycnosis of the X chromosome in the male with either heterochromatin or genic inertness.

It is the opinion of the writer that too little attention has been paid to the possibility of explaining the phenomenon of heteropycnosis on the basis of differential spiralization. There seems to be no doubt that, at times, the heteropycnosis displayed in special chromosomes, such as the Y chromosomes of Drosophila and the accessory chromosomes of maize and sorghum, is associated with a differential accumulation of thymonucleic acid on the one hand and with a comparative genic inertness on the other, but that these three phenomena are invariably and causally associated seems out of the question in the light of the evidence here presented.

It would appear unwise to build up an imposing theoretical structure expected to cover all cases of heteropycnosis on the rather meagre experimental and observational evidence at present available.

## SUMMARY

It has been demonstrated that in genera of the Acrididae, especially Melanoplus and Chortophaga, the somatic metaphase chromosomes, which have been heretofore generally described as telomitic, are really atelomitic.

The short arms of these chromosomes vary considerably in size throughout the complement and would undoubtedly furnish a valuable criterion for the identification of individual chromosomes of the haploid complement.

The view is expressed that these observations will have general significance throughout the Orthoptera.

Somatic anaphase chromosomes of Chortophaga are shown to have a simple spiral structure similar to that established in the case of plants.

By special treatment and staining, a spiral structure has been revealed in

the X chromosome of these forms, as found in the early meiotic prophase of the spermatocyte.

The spiral heretofore established in spermatogonial prophases of Tettigonidae and Acrididae and in the early meiotic prophase of the former has been described and illustrated as consisting of a thick chromonema. It has been assumed that this great thickness is due to an accumulation of thymonucleic acid generally considered as characteristic of heterochromatin. It is now shown that the apparent thickness is due largely, if not entirely, to the presence of a minor spiral upon which the major spiral is superimposed.

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# INTERRELATIONSHIPS OF THE CELLULAR CHARACTERS OF SEVERAL SPECIES OF COLUMBA[1]

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IMMUNOLOGICAL methods have so often been employed as a means of differentiating species, particularly in animals but also in plants, that it is almost a truism to state that antigenic differences are one of the more important criteria by which qualitative differences between species may be assayed. In animal species, the serum antigens have been somewhat more widely used in such comparisons than have the antigens of the tissues or blood cells. LANDSTEINER and MILLER (1925a) suggested that this was due to the presumption that the species specificities of the precipitins and of the hemagglutinins are of the same order and that species specificity means protein specificity, whereas there is definite evidence that the antigens which engender antibodies against red blood cells do not consist simply of proteins.

Various reports from this laboratory (IRWIN and COLE 1936a, 1940; IRWIN, COLE, and GORDON 1936; IRWIN 1939) have shown that, following a comparison of the relationships of the cellular characters of several pairs of species of pigeons and doves, certain general statements may be made. First, each species possessed antigenic components in common with the other and, secondly, in addition to the common substances, each species possessed cellular constituents peculiar to itself (that is, species-specific characters). The hybrids between any two species studied invariably contained all the common components of the two parental species and all or nearly all the species-specific properties of both parents. Furthermore, segregation of the species-specific characters has been observed, by virtue of successive backcrosses of the species hybrid and selected backcross hybrids to one or both parental species. Thus, in three different species crosses—Pearlneck (*Streptopelia chinensis*)×Ring dove (*St. risoria*), Pearlneck×Senegal (*St. senegalensis*), and *Columba guinea*×*C. livia domestica*, respectively—at least certain of the cellular characters peculiar to Pearlneck in the first two crosses and to *guinea* in the third have been obtained in unit form—that is, no further separation of these characters has been observed following the appropriate backcrosses.

It has also been shown, first by immunological procedures and then verified by genetical findings, that some of the cellular characters which distinguish one species (Pearlneck) from another (Ring dove) may be shared with still a third species (Senegal) (IRWIN and COLE 1940). Also, at least one, and probably two, of the antigenic substances which differentiate *C. guinea* from *C. livia*

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[2] Much technical assistance has been given in these studies by former and present Research Assistants in Genetics: DR. ALFRED GOLDEN, WARREN G. BLACK, J. R. DICK, and W. H. MCGIBBON.

are shared by *C. guinea* with both Pearlneck and Ring dove (IRWIN 1938), showing that several species may share one or more such characters to the exclusion of another species.

These and other results make highly probable the conclusion that all the antigens of the blood cells are gene-determined. Hence it can be stated with reasonable assurance that immunological studies of similarities and differences of the cells of related species will yield a fairly accurate approximation of the gross genetic relationships of such species, although hybridization may not be possible between them.

All information available at the present time leads to the conclusion that all or at least the majority of the cellular antigens are independently expressed, irrespective of the genetic complex in which each is found. If this be true, such characters should be eminently fitted for a study of the interrelationships of species.

## MATERIALS AND METHODS

In the present investigation a comparison has been made of the interrelationships of the cellular components of each of 11 species of the genus Columba, with several or all of the other ten species. These species are *Columba fasciata, flavirostris, guinea, janthina, leucocephala, livia domestica, maculosa, oenas, palumbus, picazuro* and *rufina*. (The normal habitat of these species will be given in a later report.) The comparisons of the cellular components have been made on the basis of the agglutination of the red blood corpuscles of the various pigeon species by specially prepared "test-fluids" or "reagents," as will be described below.

Antisera were produced by injecting rabbits with washed erythrocytes from representatives of each of the pigeon species. The details of these methods have been described elsewhere (IRWIN and COLE 1936a, 1936b; IRWIN 1938).

It was found in the earlier part of this work that an antiserum against the cells of any species usually agglutinated the cells of the other species of the genus at practically the same end-dilutions as were observed for the homologous cells (that is, the cells used in immunization). Therefore, such tests on the cells of the various species did not allow a definite differentiation of the cells of different species. Consequently, an antiserum against the cells of a particular species was not always tested with the cells of each of the other species. Although the data of such agglutinations are not listed in the table, they should be kept in mind as a starting point for the various comparisons. Furthermore, the agglutination of the corpuscles of these different species at approximately the same end-dilutions (usually 1:23,040 or 1:46,080) of an antiserum for any species indicates with certainty that an appreciable proportion of identical or related biochemical components was present in their respective bloods. In terms of the usual comparisons of species, these reactions would denote "homologies" in the different species.

However, a highly specific method of making a very clear cut differentiation of the cells of any two related species is possible following the absorptions of antibodies—in these experiments, the agglutinins. For example, *guinea* anti-

serum will agglutinate the cells of both *guinea* and *livia* at the same, or nearly the same, end-dilutions, but following absorption by an excess of *livia* cells, it becomes a "test-fluid," or "reagent," which will no longer agglutinate *livia* cells, even at the dilution used in the absorption (usually 1:60). However, this reagent will react with *guinea* corpuscles, ordinarily at a slightly lower titer than before the absorption (IRWIN et al. 1936). This kind of reagent, therefore, provides a highly specific and delicate test for distinguishing the cells of a pair of related species. Given such test-fluids for a pair of species, one drop of blood, even only a few bloods cells from either species may be identified with ease. As explained in previous papers (*loc. cit.*), the antibodies of an immune serum to one (the homologous) species which are absorbed by the cells of another species (as by those of *livia* in the above example) were engendered by antigens presumably common to the two species. The antibodies which are not absorbed will react with the cells of the homologous species by virtue of components "species-specific" to that species. It follows, then, that the cells of any other species which possesses cellular antigens identical with, or chemically similar to those peculiar to the homologous species (in relation to the cells of the species used in the absorption) will interact with such a reagent. Such reactions will occur by virtue of antigens which two or more species share to the exclusion of a third. This kind of reaction allows an analysis of genetic relationships of cellular characters, and therefore of their causative genes, among species hardly possible by any other technic.

Complications would of course arise in the differentiation of these or other pairs of contrasted species if, for example, there were differences between individuals within *livia* in the antigens common to these two species, and if these were detectable at the dilution of *guinea* antiserum used in making the absorptions. However, no such differences have definitely been observed in the more than 200 representatives of *livia domestica* used in these tests. Undoubtedly antigenic differences between individuals of *livia* do exist and are numerous, but our experience to date in testing for such differences in birds leads to the belief that these would be found only infrequently, with antiserums at the dilutions used in these studies of the interrelationships of species. Furthermore, the cells of several representatives of *livia*, and also of the different species among which these comparisons have been made, have usually been pooled at the various times of absorbing and testing, thereby probably eliminating any antibodies for possible antigenic differences between individuals whose cells were used in absorptions. It seems reasonable, therefore, to conclude that individual differences within the respective species probably have not appreciably influenced the interrelationships of these species, as elicited from these various tests.

The interactions of the blood cells and various reagents prepared from antiserums against the cells of these eleven species of Columba are given in table 1. Antiserums against each of the species were absorbed independently by cells of each of the other species, as far as was possible, and the respective reagents thus produced were tested against the cells of the available species. (In these tests, the deaths of representatives of certain species—particularly of *janthina*,

*leucocephala* and *palumbus*—and inability to obtain replacements have prevented the making of tests between every possible reagent and the cells of each of the species.)

The symbols used in describing the reactions given in table 1 indicate

TABLE 1

*Agglutination interactions of the cells of different species of Columba, with reagents prepared from the various antiserums to each of the species.*

| ANTISERUM | ABSORBING CELLS | TEST CELLS | | | | | | | | | | | LINE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *fasciata* | *flavirostris* | *guinea* | *janthina* | *leucocephala* | *livia* | *maculosa* | *oenas* | *palumbus* | *picazuro* | *rufina* | 1 |
| *C. fasciata* | *C. flavirostris* | C | o | + | | | ++ | ++ | C | ++ | ++ | ++ | 2 |
| | *C. guinea* | C | + | o | | | + | ++ | ++ | ± | C | ++ | 3 |
| | *C. livia* | C | ++ | + | ++ | ++ | o | ++ | ++ | + | ++ | ++ | 4 |
| | *C. maculosa* | C | ++ | o, ++ | | | ++ | o | C | C | C | ++ | 5 |
| | *C. oenas* | ++ | ++ | ? | | | ? | ++ | o | | ++ | ++ | 6 |
| | *C. palumbus* | C | ++ | ? | o, ± | ++ | + | ++ | o[1] | o | ++ | C | 7 |
| | *C. picazuro* | C | o, ± | o | | | o, + | ++ | ++ | | o | ++ | 8 |
| | *C. rufina* | ++ | o | o | | | o[2] | ++ | o, ± | (2) | o | o | 9 |
| *C. flavirostris* | *C. fasciata* | o | C | ? | | | + | ++ | + | o | ++ | C | 10 |
| | *C. guinea* | C | C | o | | | + | C | + | o | ++ | C | 11 |
| | *C. livia* | ++ | ++ | ++ | ++ | ++ | o | ++ | ± | ++ | ++ | ++ | 12 |
| | *C. maculosa* | + | C | + | | | ++ | o | + | + | ++ | ++ | 13 |
| | *C. oenas* | ++ | ++ | ? | | | ± | ++ | o | | ++ | ++ | 14 |
| | *C. palumbus* | C | C | ++[3] | | | C | C | | o | | C | 15 |
| | *C. picazuro* | + | C | o, ± | | | +[4] | +[5] | ? | | o | +[6] | 16 |
| | *C. rufina* | o, + | C | o, ± | | | o, ± | o | o, ± | (2) | o | o | 17 |
| *C. guinea* | *C. fasciata* | o | o, ± | C | C | ? | ++ | o, ± | ++ | ++ | o, ± | o, ± | 18 |
| | *C. flavirostris* | + | o | C | ++ | + | ++ | + | ++ | ++ | ± | + | 19 |
| | *C. guinea* | o | o | o | o | o | o | o | o | o | o | o | 20 |
| | *C. janthina* | ++ | ++ | C | o | ++ | C | ++ | ++ | ++ | ++ | ++ | 21 |
| | *C. leucocephala* | o | o[6] | C | ++ | o | ++ | o | ++ | ++ | o | o | 22 |
| | *C. livia* | ++ | ++ | C | C | ++ | o | ++ | ++ | ++ | + | ++ | 23 |
| | *C. maculosa* | ± | ± | C | ++ | ± | ++ | o | ++ | C | ? | ± | 24 |
| | *C. oenas* | + | o | C | ++ | + | + | + | o | ++ | ? | + | 25 |
| | *C. palumbus* | o | o[3] | C | ? | ? | ? | o | o[10] | o | o | o | 26 |
| | *C. picazuro* | o | ±[6] | C | ++ | o[7,9] | + | ? | ++ | C | o | ? | 27 |
| | *C. rufina* | o | o | C | ++ | ? | ++ | ? | ++ | ++ | o | o | 28 |
| *C. janthina* | *C. fasciata* | o | o | ++ | | | ++ | o, ± | C | C | o, ± | ± | 29 |
| | *C. flavirostris* | ++ | o | ++ | | | ++ | ++ | C | C | ++ | ++ | 30 |
| | *C. guinea* | + | + | o | | | ++ | + | ++ | ++ | ++ | ++ | 31 |
| | *C. livia* | ++ | ++ | ++ | C | ++ | o | ++ | ++ | C | ++ | ++ | 32 |
| | *C. maculosa* | C | C | C | | | ++ | o | ++ | C | C | ++ | 33 |
| | *C. oenas* | ++ | ++ | ++ | | | | ++ | o | | ++ | ++ | 34 |
| | *C. palumbus* | + | ± | o | | | ++ | o | | ? | (2) | ± | 35 |
| | *C. picazuro* | ± | o | + | | | ++ | ? | ++ | C | o | o, + | 36 |
| | *C. rufina* | ± | o | ++ | | | ++ | o | ++ | ++ | o | o | 37 |
| *C. leucocephala* | *C. fasciata* | o | ± | ++ | | | ++ | ++ | ++ | ++ | ++ | ++ | 38 |
| | *C. flavirostris* | C | o | ++ | | | + | ++ | C | ++ | ++ | ++ | 39 |
| | *C. guinea* | ++ | ++ | o | | | +[b] | +[1] | ± | o | ++ | ++ | 40 |
| | *C. livia* | ++ | ++ | ++ | ++ | ++ | o | ++ | ++ | ++ | ++ | ++ | 41 |
| | *C. maculosa* | ++ | ++ | ++ | | | + | o | ++ | ++ | ++ | ++ | 42 |
| | *C. oenas* | C | ++ | + | | | o, ± | + | o | | ++ | C | 43 |
| | *C. palumbus* | ++ | ++ | (2) | | | (2)[b] | (2)[1] | | o | C | C | 44 |
| | *C. picazuro* | + | ? | +[7] | | | + | + | ++ | | o | + | 45 |
| | *C. rufina* | o | o | ± | | | + | ± | ++ | + | o | o | 46 |

TABLE 1—(*Continued*)

| ANTISERUM | ABSORBING CELLS | TEST CELLS | | | | | | | | | | | LINE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *fasciata* | *flavirostris* | *guinea* | *janthina* | *leucocephala* | *livia* | *maculosa* | *oenas* | *palumbus* | *picazuro* | *rufina* | |
| *C. livia* | *C. fasciata* | o | ? | + | ± | ± | C | ± | ++ | ++ | ± | ± | 47 |
| | *C. flavirostris* | ++ | o | ++ | ± | + | C | + | ++ | ++ | ± | + | 48 |
| | *C. guinea* | o, + | ± | o | ? | ? | C | ± | ++ | ? | ± | + | 49 |
| | *C. janthina* | ++ | ++ | ++ | o | ++ | C | ++ | ++ | ++ | ++ | ++ | 50 |
| | *C. leucocephala* | o | o | ± | ± | o | C | o | + | ± | ? | ? | 51 |
| | *C. maculosa* | ++ | ++ | ++ | + | ++ | C | o | ++ | ++ | + | ++ | 52 |
| | *C. oenas* | + | + | ++ | o | + | C | + | o | + | + | + | 53 |
| | *C. palumbus* | o, ± | +[a] | ? | ? | ? | C | o | ++ | o | o | + | 54 |
| | *C. picazuro* | ± | o[a,a] | ++ | ± | ? | C | ± | ++ | ± | o | o, ± | 55 |
| | *C. rufina* | ±[a] | ? | + | + | ± | C | ± | ++ | ± | o | o | 56 |
| *C. maculosa* | *C. fasciata* | o | C | ± | | | + | C | ++ | o | ++ | ++ | 57 |
| | *C. flavirostris* | ++ | o | + | | | ++ | C | ++ | + | + | ++ | 58 |
| | *C. guinea* | C | C | o | | | + | C | + | ± | ++ | ++ | 59 |
| | *C. livia* | C | C | ± | + | ++ | o | C | + | ++ | ++ | C | 60 |
| | *C. oenas* | ++ | ++ | + | | | + | ++ | o | | C | C | 61 |
| | *C. picazuro* | + | o[b] | o, ± | | | + | C | ± | | o | ±[a] | 62 |
| | *C. rufina* | + | o | o, ± | | | + | ++ | + | | o | o | 63 |
| *C. oenas* | *C. fasciata* | o | o, ± | ++ | | | ++ | + | C | C | ± | + | 64 |
| | *C. flavirostris* | ++ | o | C | | | C | + | C | C | ++ | ++ | 65 |
| | *C. guinea* | ++ | o, + | o | | | ++ | + | ++ | ++ | ± | ++ | 66 |
| | *C. livia* | ++ | ± | ++ | ++ | C | o | ± | C | ++ | ++ | ++ | 67 |
| | *C. maculosa* | ++ | ++ | ++ | | | C | o | | o[g] | ++ | ++ | 68 |
| | *C. palumbus* | C[1] | + | o, +[19] | | | ++ | ② | C | o | ② | ++ | 69 |
| | *C. picazuro* | ++ | o | C | | | C | ± | C | C | o | ++ | 70 |
| | *C. rufina* | + | o | ++ | | | ++ | ± | C | C | o | o | 71 |
| *C. palumbus* | *C. fasciata* | o | o | ++ | | | ± | o[d] | ++[b] | C | o | o, ±[1] | 72 |
| | *C. flavirostris* | + | o | ++ | | | ± | o, ± | ++ | C | o, ± | ± | 73 |
| | *C. guinea* | o, ±[j] | o | o | | | o | o, ±[k] | ± | C | o | o, ±[l] | 74 |
| | *C. livia* | ++ | ± | ++ | C | ++ | o | o, ± | C | C | + | ++ | 75 |
| | *C. maculosa* | +[e] | o, ? | + | | | + | o | o[e,b,f,g] | C | ± | ± | 76 |
| | *C. oenas* | o[e] | o | ++ | | | ③ | ±[d] | o | | ± | ? | 77 |
| | *C. palumbus* | o | o | o | | o | o | o | | o | o | o | 78 |
| | *C. picazuro* | o[j] | o | + | | | ± | o[k] | ++[e] | C | o | o[l] | 79 |
| | *C. rufina* | o | o | + | | | ± | o | +[f] | C | o | o | 80 |
| *C. picazuro* | *C. fasciata* | o | ++ | o, ± | | | ± | ++ | ? | o | C | C | 81 |
| | *C. flavirostris* | C | o | ± | | | + | + | + | ++ | C | ++ | 82 |
| | *C. guinea* | C | ++ | o | | | o, ± | C | o, ± | ? | ++ | ++ | 83 |
| | *C. livia* | ++ | ++ | + | ++ | ++ | o | ++ | + | ++ | C | C | 84 |
| | *C. maculosa* | C | ++ | + | | | ++ | o | ++ | ++ | C | ++ | 85 |
| | *C. oenas* | ++ | ++ | ± | | | ++ | ++ | o | | ++ | ++ | 86 |
| | *C. rufina* | o | o | o | | | o | o | ? | ② | + | o | 87 |
| *C. rufina* | *C. fasciata* | o | + | o, ± | | | + | ++ | ? | o | C | C | 88 |
| | *C. flavirostris* | ++ | o | + | | | ± | + | C | ± | C | C | 89 |
| | *C. guinea* | ++ | ++ | o | | | + | ++ | ++ | ± | C | C | 90 |
| | *C. livia* | C | ++ | + | C | C | o | C | ++ | C | C | C | 91 |
| | *C. maculosa* | ++ | ++ | + | | | ++ | o | ++ | + | C | C | 92 |
| | *C. oenas* | ++ | C | ++ | | | ++ | C | o | | C | C | 93 |
| | *C. picazuro* | ++ | o[a] | ± | | | ± | o[a] | ++ | | o | C | 94 |

whether or not agglutination of the cells (one drop of a 2.5 percent suspension) occurred when mixed with (two drops of) a particular reagent. (Because of the small volume of reagents obtainable, the tests were usually performed at double the dilution of antiserum that was used in the absorption. If no agglutination

was noted, the mixture was repeated, whenever possible, with the reagent at the absorbing dilution.) Thus "o" indicates that no clumping of the cells could be noted by microscopical examination. A 2 indicates no clumping of cells in the second dilution of the reagent but does not preclude the possibility that there might have been agglutination in the first dilution had it been used. In some combinations the cells were not distributed entirely at random nor were there well-defined clumps. Such indefinite reactions have been labelled "?." The symbols "±," "+," "++" and "C" indicate definite agglutination, in various degrees, from numerous small clumps, usually plainly visible to the naked eye ("±"), to one large clump of cells, or complete agglutination ("C").

Each reading recorded in the table, except for those involving a few reagents prepared by the absorptions of different antisera by the cells of *oenas*, represents the results of at least two separate tests, performed at different times. Such separate tests always were made with reagents obtained by absorptions of different portions of the antiserums, rather than with the same reagents. Occasionally, at different times of testing, the same combination of reagent (from a particular antiserum) and cells produced discordant results—that is, no agglutination occurred at one test as compared with definite clumping at another. In such instances both kinds of reactions have been recorded in the table, as o, ±, etc., and may have been caused by errors in technic, by the disappearance of antibodies from an antiserum because of ageing, or by other causes.

## RESULTS

The reactions of the various combinations of reagents and cells, presented in table 1, provide information as to whether the antigens specific to one of a pair of contrasted species are shared, at least in part, by other species. For example, anti-*guinea* serum absorbed by *livia* cells agglutinates not only the cells of *guinea* but those of each of the other species as well (line 23, table 1) That is, at least a part of one or more of the antigens of *guinea*, not shared with *livia*, are found in these other species. However, it does not necessarily follow that each of these other species shares the same antigenic pattern of *guinea* which sets it and therefore, to that extent, each of them apart from *livia*.

Since most, if not all, of the specific antigens of *guinea*, not shared with *livia*, have been recognized as single characters (antigens **A**, **B**, **CD**, **E** and **F**; Irwin et al. 1936), following backcrosses of the species hybrids and selected backcross hybrids to *livia*, appropriate tests would tell which of these specific characters of *guinea* was shared, in whole or in part, with the other species. Such an analysis has been reported (Irwin 1938), showing that certain of these specific characters of *guinea* are shared with Pearlneck and Ring dove. Similar assays of these characters of *guinea* have also been done with various species of the genus Columba, and these will be reported in detail elsewhere.

## EXPLANATION OF DISCREPANCIES

As stated above, practically all the combinations of the different reagents and cells of the various species listed in table 1 have been made at least twice, many have been repeated several times. Only a few discrepant reactions have

been noted for any one antiserum to the cells of a particular species, although the reactions of reagents prepared from two or more antisera to the same species have not always agreed. That this may have been caused largely by the differential response of rabbits to immunization appears to be reasonable from the general experience in this and other laboratories (IRWIN and GOLDEN 1942).

The cross relationships of species can be tested reciprocally, and therefore very precisely, according to the principle exemplified in the following example. As stated above, *guinea* shares with each of the other species certain antigens which are not common between *guinea* and *livia*, as evidenced by the observation that the antiserum to *guinea*, when absorbed by *livia* cells, agglutinates those of the other species (line 23). Therefore, the respective antiserums to each of these species, after absorption with *livia* cells, should agglutinate the corpuscles of *guinea*. The table shows that the results expected on this basis really were obtained (lines 4, 12, 32, 41, 60, 67, 75, 84, and 91 in the column under *guinea* cells).

By far the greater majority of the reciprocal reactions of the table are in agreement for presence or absence of agglutination. A few, however, have given discrepancies on this basis of comparison. For example, although the absorption of anti-*guinea* serum by the cells of *palumbus* removed the agglutinins not only for themselves but for those of *flavirostris* as well (as shown by a lack of agglutination of *flavirostris* cells with this reagent, line 26), the antiserum to *flavirostris* after exhaustion by the corpuscles of *palumbus* reacted definitely in duplicate tests with *guinea* cells (line 15). Although the fractionation of various *guinea* antiserums did not show that this species shared any antigen with *flavirostris* which was not also shared with *palumbus*, proof of this relationship was obtained by the reciprocal tests with *flavirostris* antiserum. Thus the conclusion seems reasonably valid that the antibodies for that particular part of *guinea* were not contained in the different *guinea* antiserums used. Notations in the table, calling attention to such discrepancies in the reciprocal relationships, are marked at the pertinent combinations of the different reagents and cells, using a common superscript, in this particular comparison the superscript 3 (lines 15 and 26). The results of all the interactions of reagents and cells have been similarly examined for reciprocal agreement, and only ten such discrepancies have been observed in the many combinations of the table.

Another type of discrepancy is possible and has been found among the interactions of the cells from the different species with reagents prepared from a particular antiserum. An example of this kind may be noted in the interaction of *flavirostris* cells with reagents prepared from *livia* antiserum by absorption with the cells of *palumbus* and *picazuro*, respectively (lines 54 and 55). The corpuscles of *flavirostris* are agglutinated by the first reagent (line 54), but not by the second (line 55). These results are at variance, because the reagent prepared by the use of *palumbus* cells removes the antibodies for those of *picazuro*, whereas *palumbus* cells are agglutinated by the second reagent. Thus, of the antigens of *livia*, *palumbus* shares the same (as X, Y, and Z), and more

than does *picazuro* (as Y and Z). It would be expected, therefore, that the cells of *palumbus* would by absorption remove more antibodies from *livia* antiserum than would those of *picazuro*. Hence the cells of another species might not be agglutinated by the reagent prepared by exhausting this antiserum of antibodies for *palumbus* cells but could be clumped by the reagent produced by absorption with *picazuro* cells. The lack of agglutination of *flavirostris* cells, as shown in line 55 of the table, is therefore not in accord with expectation, since these cells are agglutinated by the reagent of anti-*livia* serum absorbed by *palumbus* corpuscles. These paired comparisons are both marked with the superscript "a." Comparable discrepancies of differences in expected results of reactions of the species cells with reagents prepared from a single antiserum are similarly marked with letters as superscripts.

The expected agreement in reactivity between cells and reagents for a particular antiserum may be stated as follows. After absorptions of any antiserum by the cells of any two or more species, if the homologous corpuscles are found to share the same components with these two or more species, the reactive capacities of their respective cells should agree when in combination with all other reagents produced from this antiserum. Furthermore, the reactions of the cells of other species toward the reagents produced by the respective absorptions of this antiserum with the corpuscles of these species should also be parallel.

On the other hand, the results may show that a particular species shares a part of its complex with one species, and this same complex plus additional antigens with another, as *guinea* shares with *oenas* all the components, and additional ones, which it has in common with *flavirostris* (line 25). Then within *guinea* antiserum there may not legitimately be a zero (0) reaction for any cells with the *flavirostris* reagent and a plus reaction for the same cells with the serum absorbed by the cells of *oenas*. Further, converse agglutinabilities should obtain for the cells of these two species—that is, cells of *flavirostris* should not react with any reagent from *guinea* antiserum which fails to agglutinate the corpuscles of *oenas*, whereas the cells of *oenas*, by virtue of the additional antigens, may readily be agglutinated by reagents which do not react with those of *flavirostris*.

### ANALYSIS OF GUINEA

In explanation of the meaning of the various tests given in table 1, it may be helpful to consider in detail the relationships of *guinea* to the other species as shown by the assay of its immune serum. One should keep in mind that the antibodies of the antiserum to a species represent what may tentatively be termed specific counterparts of the antigens of the cells of that species, so that fractionating an antiserum by the various absorptions is a means of separating the antibodies for the respective cellular antigens of a species.

Furthermore, two kinds of relationships are possible between particular antigens that are shared by any two or more species. They may be indistinguishable and therefore presumably identical, as was demonstrated by LANDSTEINER and MILLER (1925b) for the **A** and **B** characters of human blood

cells when present singly or together in the blood of anthropoid apes. Or these cellular characters in different species may be similar in structure but not identical. Examples of the latter kind have been demonstrated in lower monkeys with antigens similar to, but distinguishable from, the **B** character of human cells (LANDSTEINER and MILLER 1925c). Similarly, substances related to but not identical with either the **M** or **N** characters of humans have been found in some of the apes and lower monkeys (LANDSTEINER and WIENER 1937; WIENER 1938). Examples of antigenic similarities within the latter category have been observed in different species of birds (IRWIN, 1938; IRWIN and COLE 1940), although in these the respective antigens assayed may not have been produced by single genes, as presumably are those of humans given above. Some of the genetic implications of these relationships have already been discussed (IRWIN and CUMLEY 1940).

It is a fundamental tenet of immunology that antibodies will be engendered in an organism only to the antigens used in immunization. (A few possible exceptions to this rule appear to be satisfactorily explained on other grounds.) Therefore, the absorption of *guinea* antiserum by the cells of *guinea* and subsequent tests on the cells of the different species (line 20) are of significance. Since this absorbed serum showed no agglutination whatever with the cells of any species, we may conclude that *guinea* antiserum contained antibodies for no other antigens than those found in *guinea* cells. Results duplicating these were obtained following the absorption of anti-*palumbus* serum by the corpuscles of *palumbus* (line 78).

As stated above, the reagent produced from *guinea* antiserum by the *absorption* with *livia* cells reacts with the cells of each of the other species as well as with those of *guinea* (line 23). On the basis of these reactions, however, it cannot definitely be told whether the antigenic complexes which *guinea* shares with any two other species, as *fasciata* and *oenas*, to the exclusion of *livia*, are the same or different. This kind of assay of the different relationships requires an extension of the absorption technic and will be given in a later report. Although different degrees of agglutinative reactions to this and other reagents have been noted for the cells of certain species, it is doubtful if such differences can be considered as more than tentative indices of significance. In our opinion, the only criterion of a trustworthy differentiation is the lack of reaction of a particular reagent toward the cells of one species, as compared with definite agglutination with the cells of another.

When anti-*guinea* serum was absorbed by the cells of *flavirostris*, the reagent so produced agglutinated the corpuscles of each of the other nine species (line 19). Each of these species, then, shares with *guinea* one or more antigens not common to *guinea* and *flavirostris*. As stated above, the antigens of *guinea*, not found in *livia*, are shared in part with *flavirostris*. *Guinea* therefore shares some antigens with *livia* that are not found in *flavirostris* and some with *flavirostris* that are not held in common with *livia*, and, presumably, *guinea* shares many with both *flavirostris* and *livia*. (For somewhat comparable relationships, see the diagrammatic representations of the antigens of *guinea*, *livia*, Pearlneck and Ring dove, IRWIN 1938.)

Exhaustion of anti-*guinea* serum with the cells of *janthina* and *maculosa*, respectively, provided reagents which also agglutinated in various degrees the cells of the other species (lines 21 and 24), except those used in the individual absorptions. These results therefore parallel those obtained with the reagents produced in this antiserum following independent absorptions by the cells of *livia* and *flavirostris*, except that a different species is involved in each comparison with *guinea*. Unfortunately the relationships of *guinea* to these four species, as well as to the others, cannot be accurately diagrammed from the data presented. That is, from the results obtained with the reagent produced by exhausting *guinea* antiserum with cells of *flavirostris*, for example, no statement can be made as to whether the agglutinations of the cells of any two species, as *fasciata* and *leucocephala*, are by virtue of the same or different characters. But, on the basis of the reactions observed following fractionation of the antiserum to *guinea* cells by these four absorptions, it can be reasonably concluded that the antigens which engendered these antibodies must have been numerous.

The reagent prepared by absorbing *guinea* antiserum with the corpuscles of *oenas* agglutinated the cells of each of the other species except *flavirostris*, although it produced only a faint trace of agglutination, if any, with the corpuscles of *picazuro* (line 25). Since there is agglutination of the cells of *oenas* following absorption of this antiserum by those of *flavirostris* (line 19), as stated above, it appears that *guinea* shares with *oenas* the same and more substances than are shared with *flavirostris*. *Guinea* cells are therefore undoubtedly more closely related in antigenic structure to those of *oenas* than to those of *flavirostris*. Thus, the cells of *oenas* will be expected always to be agglutinated by the different reagents prepared from *guinea* antiserum, which clump those of *flavirsotris; oenas* cells may also be agglutinated in combinations with reagents which do not clump *flavirostris* corpuscles. According to the principles stated above, this latter reaction is possible by virtue of the antigens of *guinea* shared with *oenas* but not with *flavirostris* (for examples, see lines 22 and 28).

A slightly different picture of species relationships may be noted from the results of tests with reagents obtained by the respective absorptions of *guinea* antiserum with the cells of the remaining species. Following absorption with *fasciata* cells (line 18), the test fluid agglutinated strongly the cells of *guinea*, *janthina*, *livia*, *oenas*, and *palumbus*. Discrepancies in results have been observed for this reagent with the cells of *flavirostris*, *maculosa*, *picazuro*, and *rufina*, in that no agglutinations, in contrast to only a relatively few clumps of cells, have been noted for the cells of each of these species at different times of test. It is probable, however, that *guinea* shares with each of these latter species a very minute fraction of its cellular antigens not common to *fasciata*. The suspicious reaction of this reagent (*guinea* antiserum absorbed by the cells of *fasciata*) with the cells of *leucocephala* has been repeatable and therefore presumably represents a definite but minute antigenic similarity of *guinea* and *leucocephala* to the exclusion of *fasciata*. This relationship is verified

by the reciprocal test—that is, agglutination of *guinea* cells by *leucocephala* antiserum absorbed by *fasciata* cells (line 38).

Following exhaustion of *guinea* antiserum by the cells of *picazuro*, no clumping was noted with the corpuscles of *fasciata* or *leucocephala*, and only suspicious reactions with those of *maculosa* and *rufina* (line 27). The antibodies were removed in this absorption for the cells of *fasciata* and *leucocephala* as well as for the absorbing cells. However, the reciprocal test of *leucocephala* antiserum absorbed with *picazuro* cells (line 45) showed definite agglutination of *guinea* cells (discrepancy 7). It is therefore probable that the *guinea* antiserums, from which these reagents have been derived by exhaustion with cells of *picazuro*, were deficient in the antibody specific for that part of the pattern shared with the cells of *leucocephala* to the exclusion of *picazuro*. (The possibility of a non-specific absorption of the antibody cannot be entirely excluded in explanation of the discrepancy.)

Since absorption by *rufina* cells removed from *guinea* antiserum the agglutinins for *picazuro* cells and for those of *flavirostris* as well (line 28), it may be concluded that all the cellular antigens which *guinea* shares with *flavirostris* and *picazuro* are also shared with *rufina*. It is probable, also that *guinea* and *rufina* have a small fraction of antigenic components in common to the exclusion of *picazuro* (compare reactions of cells and reagents in lines 27 and 28). Furthermore, since *rufina* cells by absorption likewise remove the antibodies from *guinea* antiserum for the corpuscles of *flavirostris* but not for those of *oenas* there is pertinent evidence for the statement that *guinea* shares a complex of antigens with the three species, *flavirostris*, *oenas*, and *rufina*. In addition to the complex held in common with these three species, *guinea* shares other characters with *oenas* and still others with *rufina*. *Guinea* is therefore more closely related to both *oenas* and *rufina* than to *flavirostris*, but these tests do not allow a statement concerning any possible difference in the degree of relationship of *guinea* to either *oenas* or *rufina*.

Following absorption of *guinea* antiserum with the cells of *leucocephala* (line 22), no antibodies remained for either *picazuro* or *rufina* corpuscles, nor for those of *maculosa*. Naturally, antibodies were also removed for the cells of any species which had less of *guinea* substances than any one of these—that is, *fasciata* and *flavirostris*. On this basis, *guinea* is more closely related to *leucocephala* than to *rufina*, *picazuro*, *fasciata*, *flavirostris*, or *maculosa*. The data, however, do not permit a statement as to what extent *guinea* shares the same antigens with *oenas* and *leucocephala*, respectively.

Finally, when the antiserum to *guinea* was absorbed by the cells of *palumbus*, only suspicious reactions were observed with the cells of *janthina*, *leucocephala*, and *livia*, and none with those of the others except with the homologous cells (line 26) and possibly with *flavirostris* (discrepancy 3). These particular results show that *guinea* shares with the other nine species very few, if any, of its cellular antigens not common to *palumbus*. Reciprocally, *guinea* cells exhaust *palumbus* antiserum (line 74) of antibodies for the cells of *flavirostris*, *livia*, and *picazuro*, and leave antibodies capable of reacting at the first dilution only

weakly with the corpuscles of the other species. *Palumbus* cells, however, were agglutinated strongly by this latter reagent. Thus it seems reasonably definite that *guinea* and *palumbus* are more closely related to each other in these cellular characters than to any other species of those tested.

The logical conclusion to be drawn from the fractionation of *guinea* antiserum, as described above, is that the cellular characters of *guinea* form a complex pattern within which are woven interlocking relationships with each of the other ten species. Just how these antigens of *guinea* are interwoven among the other species cannot be determined precisely from the data here presented, but are susceptible to experimental assay, as will be described elsewhere.

## GENERAL RELATIONSHIPS

The antiserum for each species might be analyzed in detail in the same manner as has been done above for *guinea* antiserum. From such analysis would emerge a picture of the antigens of each species interlocking in intricate but somewhat dissimilar patterns with those of the others. In general, the interrelationships of the cellular characters of one species to those of the others may be divided into several reasonably well defined groups, as follows:

(1) The antiserum to one species, when absorbed by the cells of another, may still react in various degrees with the corpuscles of all the other species. For example, anti-*guinea* serum when absorbed with *livia* cells reacted with the cells of each of the other species. Likewise, the antisera to each of the other species (excluding *livia* antiserum), when exhausted by *livia* cells (with but one possible exception—line 75), agglutinated the cells of all other species. Thus the antigens of one species, not shared with another, may be shared at least in part with all the other species.

(2) Absorption of an antiserum by the cells of another species may remove antibodies not only for the absorbing cells, but for those of one, two, three, or more other species. That is, a given species may share (a) a complex of antigens with one species, it may share (b) either the same complex or this same complex plus additional antigens with another species, or (c) all the antigens of the second species plus others with a third, etc. Different degrees of relationships between species may be seen in the data of the table.

Thus anti-*fasciata* serum, when absorbed by *guinea* corpuscles, agglutinated in varying degrees the cells of the ten other species (line 3). *Fasciata* then shares cellular antigens, but not necessarily the same antigens, with each of the other species which it does not share with *guinea*. Furthermore, when *fasciata* antiserum was exhausted by the cells of *picazuro* (line 8), no reaction was obtained between the reagent so produced and *guinea* corpuscles. Therefore, *fasciata* possesses in common with *picazuro* all and more cellular antigens than are shared with *guinea*. Similarly, since the reagent produced by absorption of *fasciata* antiserum by *rufina* corpuscles (line 9) failed to agglutinate either *guinea* or *picazuro* cells (as well as those of other species), whereas *rufina* cells are agglutinated by each of the other reagents produced from this antiserum, it seems certain that *fasciata* cells in common with those of *rufina*

have all the antigens which are shared with *picazuro* and *guinea*, plus others in addition.

(3) It is conceivable that two species could be so closely related that neither would share with any other species the cellular characters which distinguish the one from the other. No examples of this kind are to be found in the data of table 1, although, as cited above, *guinea* and *palumbus* are very closely related. A slightly different picture of antigenic relationships may be seen for *picazuro* and *rufina*. *Picazuro* shares practically no cellular characters with any other species that are not shared with *rufina* and seemingly has only a small proportion of antigens particular to itself (line 87). On the other hand, at least some of the antigens of *rufina*, not in *picazuro*, are shared by *fasciata* and *oenas*, and a minute fraction by *guinea* and *livia* (line 94). Therefore, it appears that *picazuro* and *rufina* are very closely related, but that *picazuro* has less of antigens specific to itself than has *rufina*.

The question naturally arises as to whether the antigenic components, and therefore the causative genes, of one species may simply be the sum of those found in several related species. That is, does any one species have cellular characters which are particular to that species alone and are not found in any other? It is conceivable that some species may possess genes with antigenic effects quite unlike those of any combination of other species, while most, if not all, of the antigens of others may well be nothing more than the sum of the components of certain combinations of related species. This question will be considered further in future reports.

## OTHER ANALYSES

Another step in the analysis of the interrelationships of the antigenic characters of these species that can be made would be to determine if there are any parallel reactions of the various species' antiserums, when each is separately absorbed with the cells of a single species and then tested with the cells of each of the other species. That is, do the reactions of the respective antiserums thus absorbed, toward the corpuscles of all the various species, suggest a common complex of antigens between any two or more species which are shared to the exclusion of a single species? Such comparisons may very easily be made by a rearrangement of the data of table 1, listing together the reactions of the various antisera, following absorption by the cells of a single species, toward the cells of each of the different species.

An example of this kind may be noted in the reactions of antiserums to *flavirostris*, *maculosa*, *picazuro*, and *rufina*, respectively, following the absorptions of each by the cells of *fasciata* (compare lines 10, 57, 81, and 88). Except for differences in the degree of reactivity of these four reagents toward their homologous cells, their reactions toward the cells of other species are very similar. There were relatively weak or uncertain agglutinations of the respective reagents with the cells of *guinea* and none with those of *palumbus*. Thus, these four species share no antigens with *palumbus*, and very few with *guinea*, that they do not possess in common with *fasciata*.

Substantiating evidence of the statement made earlier that *guinea* and

*palumbus* are closely related in their content of cellular antigens may be derived from the interactions with the cells of either *guinea* or *palumbus* to reagents produced by absorption of antiserums to the remaining nine species by the cells of the other. When the absorptions were done with *guinea* corpuscles, the reactions for *palumbus* cells were completely wiped out in the antiserums from *flavirostris* and *leucocephala*, with faint if any reactions with those from *livia*, *maculosa*, *picazuro*, and *rufina*. Exhaustions of the antiserums to *picazuro* and *rufina* were not done with *palumbus* cells; the other antiserums, except that to *flavirostris*, when exhausted by *palumbus* cells gave only questionable, if any, reactions with *guinea* corpuscles.

A similar substantiation of the close relationship proposed previously between *picazuro* and *rufina* will be obtained if the data of table 1 are examined in like manner. The cells of *picazuro* exhausted the respective antiserums appreciably, but not entirely, of the antibodies for *rufina* corpuscles, while exhaustion of the different antisera by the antigens of *rufina* in every instance removed also the antibodies for *picazuro* cells. Thus the conclusion seems valid that whereas *picazuro* and *rufina* both share antigenic components with the other species, in every comparison of these two with a third species, *rufina* shares all and usually more cellular components than does *picazuro*. These results agree entirely with the statements made above, that *rufina* has more cellular components specific to itself than does *picazuro*.

Furthermore, the reagents produced by absorptions of these different antisera by *picazuro* cells (except in anti-*flavirostris* serum) reacted only faintly, if at all, with *flavirostris* cells. Results paralleling these were obtained following the absorptions of the same antiserums with the corpuscles of *rufina*—that is, no reaction of *flavirostris* cells except with this type of reagent from the homologous antiserum. The reactions for the corpuscles of *fasciata* were generally faint, if any existed at all, with the reagents obtained by absorptions of the respective antiserums by the corpuscles of either *picazuro* or *rufina*.

There are many other ramifications of the relationships of the species that could be discussed in detail. Perhaps the most important single conclusion that can be drawn from an analysis of these antigenic relationships is that each of the species studied, in its interrelationships with the others, appears definitely to be an entity. How far variation between individuals within any of these species may be found to reduce the distinction between the species is an open question. It must be admitted that the number of representatives from these different species which were available for testing was extremely small, and the hazards of extrapolation from these few to the species as a whole should not be minimized. However, as stated above, we question, in the light of our experience, whether differences in the antigenic composition of individuals would materially change the results of the relationships reported here, particularly at the level (dilution of antiserums) at which the tests were made.

For example, two recently imported birds of the *picazuro* species were obtained early in 1937 from a dealer in California. Later, two more birds of this species were provided by courtesy of Drs. Holmberg and Roselli of the Zoological Gardens of Buenos Aires. Still later, 11 of these birds, coming

from two recent importations from South America, were purchased from a dealer in Maryland. Although not all the tests involving absorption by, or agglutination of, *picazuro* cells were repeated for these different samples of the species, in no case in which duplicate tests were made was there a suggestion of different reactivities.

Another general conclusion is that there appears to be a reasonably well-pronounced tendency for certain of these species to resemble each other more than others. Thus *fasciata*, *flavirostris*, *maculosa*, *picazuro*, *rufina* and, insofar as the data show, *leucocephala* appear to be more closely interrelated each to the others than they are to *guinea*, *livia*, *oenas*, *palumbus*, and probably *janthina*, and vice versa. Since these groupings coincide with the native habitat of these different species in the Old and New Worlds, respectively, there may well be for these species a significant correlation of geographical habitat with evolutionary progression. A paper to follow will discuss these relationships.

Although the evidence points strongly to the conclusion that the gene complexes affecting the cellular characters of these species may be roughly divided into two primary groups, it seems reasonable to conclude from the above tests that the characters common to any two of the species are probably more numerous than are those which are particular to either of the species. As stated above, the cells of these different species very seldom, if ever, are clearly differentiated by their reactivities with untreated antiserum against any species—that is, each antiserum agglutinates the homologous cells and those of the other species to approximately the same end-dilutions. The logical inference to be drawn from such interactions is that a species shares a considerable proportion of its cellular antigens with all these other species. It is not unreasonable to assume that a goodly proportion of the characters common to two species are also common to all the species.

Granted that an accurate estimate cannot now be reached of the number of characters shared by any two of these species, and even less of those probably shared by all, nevertheless an approximation may be made of the relative proportion of the chromosomes of *guinea* which bear genes making for common and species-specific cellular characters, respectively, in contrast with *livia*. From such information, it is possible to postulate a somewhat similar relationship between other pairs of species. It was previously shown (IRWIN et al. 1936) that there were six cellular characters (**A, B, C, D, E,** and **F**) which differentiate the blood cells of *guinea* from those of *livia*. At the present writing only five of these are available in unit form; antigen **C** has not been perpetuated singly. These characters have segregated in the offspring of the backcrosses to *livia* of the species hybrids between *guinea* and *livia*, and selected backcross hybrids. Each of these *guinea* components is assumed to be produced by the action of one or more genes on a chromosome in *guinea;* unless there is a linkage between some of these genes, there are five or six chromosomes of *guinea* which produce "major" cellular antigens, specific to *guinea*. (It is entirely possible, of course, that any one or all of these five or six chromosomes may also carry genes which produce effects common to the two species, as well as particular to *guinea*.) These are called major characters because they

are expressed at relatively high dilutions of the absorbed antiserum; **A** and **F** usually are agglutinated at end-dilutions of 1:180 or 1:360, and **B, D,** and **E** ordinarily react at end-dilutions of 1:1440, often as high as 1:5760. It is, of course, conceivable that there may also be "minor" characters peculiar to *guinea*, but these supposedly would be expressed only at lower dilutions (less than 1:60) of *guinea* antiserum than have been used in these tests. Therefore, except that there may be minor characters specific to *guinea*, any other genes in *guinea* producing antigenic effects in the red blood cells should produce components shared with *livia*. (It is possible, in fact probable, that the so-called "major characters" of *guinea* may individually be the result of joint effects of several genes on the respective chromosomes. Since it has been shown (Irwin 1938) that qualitatively different component parts of the **CD** and **E** characters of *guinea* may be shared by Pearlneck and Ring dove, respectively, to the exclusion of *livia*, it appears rather improbable that these antigens are each produced by a simple gene, or by the action of the individual chromosomes as a whole in *guinea*, as proposed in essence by GOLDSCHMIDT (1940).

Evidence applying rather directly to this point may be adduced from the results of experiments on the number of cellular antigens within a species. The greatest number of these known at the present writing for any species has been found in this laboratory in the cells of cattle (FERGUSON 1941; FERGUSON et al., 1942). The 30 cellular antigens now recognized in cattle furnish substantiating evidence for the proposal that one or more genes on each of the chromosomes of a species may have effects on the antigens of the blood cells. (The work of LANDSTEINER and LEVINE (1932), TODD (1930), and unpublished results from this laboratory indicate very strongly that a similar situation holds for the cells of the chicken.) If this be true, and if, therefore, one or more genes on each of the presumed 30 pairs[3] of chromosomes of *guinea* have such effects, the genes on only five or six of these serve to distinguish *guinea* from *livia*. Presumably all the other antigens of *guinea* would be shared by the two species, although, as stated above, there might be "minor" antigens, not expressed at the level of the recorded tests, which would be species-specific to *guinea* but not readily detectable. That is, it is entirely possible that one or more genes on each of the probable 24 other chromosomes of *guinea*, than these five or six which produce specific *guinea* characters, initiate antigens common to both *guinea* and *livia*.

Each of the cellular characters which differentiate *guinea* from *livia* has so far behaved as a unit in inheritance and might therefore be construed as being determined by a single gene. On such an interpretation, there would be the somewhat anomalous situation in which the erythrocytes of *guinea* differed from those of *livia* (a different species) in the effects of only six genes, whereas the cells of individual cattle (the same species) theoretically may differ by the

[3] Actual counts of the chromosomes of *guinea* have not been made. On the supposition that the chromosome numbers of the species of Columba and Streptopelia would be very similar, we are assuming that these species of Columba have the same number as *livia* and Ring dove—that is, approximately 30 pairs (unpublished data by T. S. PAINTER, personal communication).

effects of as many as 30, since no two of the causative genes seem to have a simple allelic relationship. (Actually, however, the lowest number of known antigens observed in the cells of any individual in cattle has been four or five, while the greatest number has been rarely, if ever, above twenty. Furthermore, nearly all the cellular antigens recognized in cattle might be classed as "minor" characters. That is, they are reactive at dilutions of their respective reagents much lower than those which have detected the five or six "major" characters specific to *guinea*.) It therefore seems more probable that each of the five or six cellular antigens specific to *guinea* is produced by two or more genes on the individual chromosomes.

Furthermore, any species which possessed all the cellular characters which are common to *guinea* and *livia*, as well as a part of the components specific to *guinea* in contrast with *livia*, would certainly differ from *guinea* only to the extent that it did not share the *guinea* specific substances *in toto*. Only *palumbus* appears to have in common with *guinea* practically all the components shared between *guinea* and *livia* (line 26) as well as a part of those specific to *guinea*, in contrast to *livia*. Unpublished evidence suggests that *palumbus* contains all the specific *guinea* characters **A** and **F**, but only a part of **CD** and **E**, and no more than a minute fraction, if any, of **B**. Hence *guinea* differs from *palumbus* in the biochemical composition of its erythrocytes in the effects of genes on three or four chromosomes—namely, those with genes affecting, respectively, character **B** and parts of characters **CD** and **E**,

Although there is definite evidence that the blood cells of *guinea* differ from those of *livia* in five or six major antigens, and by inference differ from those of *palumbus* in three characters, each determined by one or more genes on as many chromosomes, there is no reason to assume that the chemical differences between these species are limited to these cellular characters or to the effects of the genes causing them. Recent results have shown a segregation of antigens specific to the serum of one of the parental species, in backcross individuals from each of three different species crosses (CUMLEY, IRWIN and COLE, 1941; CUMLEY and IRWIN, 1942, unpublished data; IRWIN and CUMLEY 1942). The species-specific constituents of the serum have separated independently of those of the cells in each of the four kinds of backcross progeny, implying independent and specific action of the genes in each species producing the species-specific effects in the cells and serum, respectively. Hence in *guinea* it is reasonable to assume that chromosomes other than those which carry genes producing the five or six *guinea* specific characters of the cells may have genes influencing the chemical composition of the serum (proteins). Whether the chromosomes of *guinea*, carrying genes for any kind of biochemical differentiation of that species from any other, will also carry genes affecting other characters distinguishing that species is still an open question. Could this be answered, it would undoubtedly provide information as to the relative importance of the various kinds of characters by which differentiations of species are attempted.

All these comparisons of species relationships have been made herein on the basis of characters which definitely distinguish one species from another (that

is, species-specific characters). Also, an extension of the comparisons has been made to determine whether the specific characters of one species are shared, at least in part, with still other species of the genus. The emphasis is therefore placed first of all on the differences between a pair of related species, with the underlying assumption, based on a reasonable amount of experimental evidence, that the differences as well as the resemblances are genetically determined. Moreover, in the light of our present knowledge, the genes responsible for the cellular characters appear to produce their effects irrespective of the total genetic complex. Thus, effects of both external and internal environment on these characters are presumably at a minimum.

In some respects this kind of biochemical assay of relationships between species differs slightly from the general picture of relationships obtained by the methods of either classic taxonomy or cytology. ANDERSON (1937) states that "cytology, or more properly karyology, concerns itself with the architecture of the germplasm; taxonomy with the adult forms which result from germplasms." In general, these two disciplines deal primarily with homologies between species, whether the homologies are in the nature of a morphological character or in the banding of a salivary chromosome. The examination of the salivary gland chromosomes of various species and strains of Drosophila has resulted in a picture of differences in gene rearrangements, or inversions, in these forms. Such studies are somewhat comparable, then, to these biochemical studies of differences between species. For example PATTERSON (1942) has compared, among others, five species of the group of *D. virilis* and has proposed that one species, *americana*, evolved from hydrids between *novamexicana* and *texana*. Although STURTEVANT (1942) has questioned the specific status of certain members of the *virilis* group, they serve to emphasize the well-recognized fact that it is possible that gene rearrangements have played a significant role in the evolution of species. Similar studies have been made by several workers on populations of races A and B of *D. pseudoobscura*, as a result of which a phylogenetic chart of relationships has been constructed (DOBZHANSKY 1941). Should it be found that gene rearrangements are accompanied by biochemical changes, their probable role in evolutionary processes could hardly be questioned. It has already been shown (CUMLEY 1940) that grouping of Drosophila species by serological methods corresponds relatively closely to the grouping by the use of morphological characters. In our opinion, these methods may be employed to determine whether the various kinds of gene rearrangements of themselves produce biochemical changes.

In conclusion, the experimental evidence from all species crosses, from which an assay of the segregation of antigens has been made, indicates strongly that the biochemical characters of the blood cells of such pigeon and dove species by which one species is differentiated from another appear to be produced by the action of one or more, probably usually of several, genes on each of a relatively small proportion of the chromosomes of the respective species, rather than by genes scattered over most of the chromosomes. In our opinion, parallel relationships obtain between the various species reported in this paper, although species hybrids and backcross hybrids have not yet been

obtained between them. These findings suggest that at least the major changes of this kind, which have taken place in the germinal material, have been confined to a few chromosomes. Eventually it should be possible to assay the chemistry of these or comparable characters, presumably thereby gaining some knowledge of the structural changes (DOBZHANSKY 1941, p. 85) that must have occurred in genes during the evolution of species. Such a statement is based on the supposition, as previously proposed (IRWIN and COLE 1936a; HALDANE 1938) that the cellular antigens are more or less primary products of their causative genes.

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# TESTS FOR RECOMBINATION AMONGST THREE LETHAL MUTATIONS IN THE HOUSE MOUSE[1]

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IN THE mouse a peculiar relation exists amongst three lethal mutations which produce similar effects on development. Animals which inherit two doses of the same lethal die as early embryos, while those which inherit one dose each of two different lethals of this group may be viable. This is the result to be expected when the lethals involved occupy different loci, but in the present case the different lethals have shown no recombination with each other and may therefore be considered either as unilocal or as occupying different loci amongst which crossing over is prevented.

If the first alternative were proved to be correct, there would be revealed a type of complementary interaction amongst alleles which could not be reconciled readily with theories which regard changes at the same locus as purely quantitative in character. It is a fact, however, that the mutations in question have certain other effects in common and thus resemble other series of multiple alleles, the members of which appear to differ in quantity of effect.

The first step in resolving this contradictory situation is to determine whether or not the mutations involved are unilocal. In the present paper the question will be examined as to whether or not any recombination occurs in animals heterozygous for different members of this group of lethals.

## MATERIAL

The mutations used were first found and described by DOBROVOLSKAIA-ZAWADSKAIA and KOBOZIEFF (1927, 1932). The genetical analyses upon which the current interpretations rest were carried out by CHESLEY and DUNN (1936), DUNN (1937 and 1939), and DUNN and GLUECKSOHN-SCHOENHEIMER (1939). The theory proposed by the above workers has been independently confirmed by KOBOZIEFF and POMRIASKINSKY-KOBOZIEFF (1939a, 1939b). In brief it rests upon the behavior of three lines of tailless mice, each of which breeds true as a balanced lethal system. One component of this system is the same in all lines. This is the Brachyury mutation $T$ which when heterozygous ($T+$) produces a short tail and when homozygous produces characteristic abnormal embryos which die before birth. In Line A, this is combined with a recessive $t^0$, which when homozygous acts as an early lethal, and in combination with $T$ produces taillessness ($Tt^0$). Line 29 ($Tt^1$) is similarly tailless, $t^1$ exerting its lethal effect before implantation of the embryo. Line 19, a true-breeding tailless line, behaved in all respects like Line 29 and was probably $Tt^x$, $t^x$ having arisen as a recurrence of $t^1$. From matings within Line A ($Tt^0$ by $Tt^0$) the only viable zygotes recovered are $Tt^0$; recombination gametes

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lacking both $T$ and $t^0$ are not found. Similar reasoning applies to the balanced lethal behavior of Line 29 and Line 19. The proof of absence of recombination depends of course on the number of individuals observed which could have been detected as recombinants, and these data, derived from balanced lethal lines, will be presented first.

Secondly, the results of crosses between tailless animals from the balanced lines and individuals lacking one or both mutant factors will be given as tests of recombination in outcross progeny.

Thirdly, the evidence from crosses between members of different balanced lines will be examined. One of the genotypes produced by such crosses was interpreted by DUNN (1937) as a viable compound between two different lethal alleles ($t^0t^1$), one from each of the balanced lines. Since this type yields the major part of the recombination data, it has been studied in detail. It proved to be a type which suffers a high mortality before birth; and in the course of embryological observations which this fact required us to make, a new type of abnormality associated with the $t^0t^1$ genotype was discovered. These observations provided the clue to an interpretation of some of the puzzling ratio variations encountered in crosses between different balanced lethal lines. Without such an interpretation, the data on segregation would be subject to a degree of uncertainty which would preclude their use in a study of crossing over.

## THE ABSENCE OF RECOMBINATION WITHIN THE BALANCED LETHAL LINES

The balanced lethal lines have maintained themselves without selection for more than ten years and have behaved similarly in each of the laboratories in which they have been bred (COLUMBIA UNIVERSITY and INSTITUT CURIE). While this is evidence that the lethals "balance," it does not by itself prove that crossing over does not occur. From parents $Tt^1$ by $Tt^1$ in which the non-crossover gametes are $T$ and $t^1$, crossover gametes $++$ would be detectable when in combination with regular gametes $T$ ($T+$ = Brachy) or $t^1$ ($+t^1$ = normal). The reciprocal type of crossover gamete ($Tt^1$) would fail to survive in combination either with $T$ or $t^1$. Hence half of the crossover gametes should be detectable. A careful watch has been kept for such exceptional young.

Table 1, however, shows that no recombinations have been found among over 2900 offspring observed. Within any one line all offspring tested have been shown to have the same genotype for $T$ and $t^0$ or $t^1$. We have noted, however, some phenotypic variation. Thus occasional animals of Line 29 when bred on a Bagg albino genetic background had a small bony stump tail. After reconstitution of this line following an outcross to several normal stocks, the frequency of such stump-tailed animals was noticeably increased, and a few animals with short tails up to one third of the normal length were found. Nearly all these exceptions occurred in one family which appears to contain factors for increased tail development in animals of genotype $Tt^1$. Such exceptional animals as have been tested (four males and four females) breed as do the other members of the line—that is, with tailless mates they produce either tailless, stump, or short-tailed offspring (41 tailless, 15 stump, 8 short-tailed).

Clearly they tend to transmit their exceptional phenotype while maintaining their balanced lethal behavior.

TABLE 1

*Results of matings within balanced tailless lines; offspring classified on day of birth.*

| TAILLESS PARENTS | OFFSPRING | | | TOTAL CLASSIFIED |
|---|---|---|---|---|
| | TAILLESS | STUMP | SHORT | |
| Line A×Line A[1] | 573 | | | 573 |
| Line 29×Line 29[2] | 549 | 13 | | 562 |
| Line 19×Line 19[3] | 85 | | | 85 |
| Line A×Line A[4] | 458 | 23 | 3 | 484 |
| Line 29×Line 29[4] | 537 | 23 | 10 | 570 |
| Line A×Line A[5] | 63 | | | 63 |
| Line A×Line A[6] | 329 | | | 329 |
| Line 29×Line 29[5] | 194 | | | 194 |
| Line 29×Line 29[6] | 116 | | | 116 |
| Totals | 2904 | 59 | 13 | 2976 |

[1] Tailless Line A backcrossed and extracted from inbred Bagg albino stock.
[2] Tailless Line 29 backcrossed and extracted from inbred Bagg albino stock.
[3] Tailless Line 19 backcrossed and extracted from inbred Bagg albino stock (DUNN 1939).
[4] New lines extracted from outcrosses.
[5] From KOBOZIEFF 1935.
[6] From KOBOZIEFF et al. 1939.

The same statements may be made concerning the exceptional animals which appeared in Line A after recovery of this line from an outcross.

Although not all exceptional animals have yet been tested, it is safe to say that the short-tailed animals which appear in certain tailless families are in general not the result of recombination.

### TESTS FOR RECOMBINATION GAMETES IN OUTCROSSES OF TAILLESS ANIMALS

If no recombination occurs between $T$ and $t^1$ (or $T$ and $t^0$) then when $Tt^1$ (or $Tt^0$) animals are crossed with normal ($++$) or Brachy ($+T$), all $F_1$ normal-tailed offspring should be $+t^1$ (or $+t^0$) without exception. Crossing over between $T$ and $t^1$ would result in the production of $++$ gametes, hence in $F_1$ normals which do not contain $t^1$ (or $t^0$).

Progeny tests of $F_1$ normal-tailed animals have been carried out in a search for such exceptions. The test was to cross the $F_1$ animal to Brachy ($+T$). If $t^0$ or $t^1$ is present in the $F_1$, then tailless progeny ($Tt^0$ or $Tt^1$) appear. As table 2 shows, this occurred in the case of 378 $F_1$ animals, and to this we should add 33 tested offspring from Line 19 tailless (DUNN 1939). It did not occur in progenies produced by three $F_1$ animals, but since these progenies consisted of only 2, 4, and 4 normal offspring, respectively, it is clear that the absence of tailless offspring is probably due to the small samples and that no valid exception occurred.

We may conclude that there is no evidence of crossing over between factors at locus $T$ when tested within balanced lines or in outcrosses.

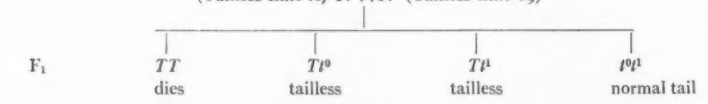
TABLE 2

*Distribution of $F_1$ normal tailed offspring from tailless which were individually tested and found to be $+t^0$ or $+t^1$.*

| PARENTS ♀ | PARENTS ♂ | TESTED NORMAL TAILED OFFSPRING ♀ | ♂ | ♀ or ♂ | |
|---|---|---|---|---|---|
| Tailless A | Normal | 22 | 7 | | |
| Tailless A | Brachy | 1 | 1 | | |
| Normal | Tailless A | 117 | 27 | | |
| Brachy | Tailless A | 24 | | | |
| Tailless 29 | Normal | 15 | | | |
| Tailless 29 | Brachy | | 1 | | |
| Normal | Tailless 29 | 120 | 25 | | |
| Brachy | Tailless 29 | 16 | 2 | | |
| Normal | Tailless 19 | | | 33 | |
| | Total tested | 315 | 63 | 33 | 411 |

CROSSES BETWEEN DIFFERENT TAILLESS (BALANCED LETHAL )LINES

It had been shown by KOBOZIEFF (1935) that crosses of tailless animals of Line A by tailless animals of Line 29 produced normal-tailed offspring in addition to tailless ones. DUNN (1937) explained these and similar results of his own by assuming that the recessive lethals in Lines A and 29 were different and that the compound containing different recessive lethals was viable and normal-tailed. The scheme proposed is reproduced in figure 1.




The first results obtained conformed qualitatively to the above scheme, and in tests of the $t^0t^1$ type no recombination between $t^0$ and $t^1$ was observed in 155 test gametes, suggesting that $t^0$ and $t^1$ were alleles. All males of this genotype which were thoroughly tested proved to be sterile. These observations were too few to constitute a rigid test of the theory proposed, and the ratio of tailless to normal-tailed animals obtained (81:41), while in apparent agreement with that expected from the theory, was actually at variance with a fact which was proved in other experiments—namely, that $Tt^0$ and $Tt^1$ males always produce a considerable excess of offspring which inherit $t^0$ or $t^1$. New experiments were undertaken to investigate this point.

Reciprocal crosses between tailless animals of Lines A and 29 were made and simultaneous test crosses of Line A tailless males by Line 29 tailless females and by normal females. The general low fertility and poor viability of tailless animals delayed completion of the experiment for several years. The same circumstances forced us to resort to crosses of $+t^1$ (normal) females by

$Tt^0$ males and of $+t^0$ females by $Tt^1$ males. From these crosses further $t^0t^1$ females were obtained for the crossing over tests.

The combined results of crosses between tailless animals of Line 29 and tailless animals of Line A are shown in table 3. Here the results fit the theory in so far as only two classes of offspring appear—that is, tailless and normal-tailed. This fact shows that recombination between $T$ and $t^0$ or $T$ and $t^1$ probably does not occur, since the recombination gamete $++$ when uniting with $T$ from the other parent would produce Brachy offspring $+T$. Out of 1208 offspring observed three were classed as Brachy at birth. One of these with a tail one-third of normal length died before it could be tested. Another with a tail one-fourth of normal length proved to be $Tt^0$, since when mated with $Tt^0$ it produced only tailless (7), and when crossed with $Tt^1$ it produced only normal and tailless (12:28). The latter is conclusive evidence that it was not $T+$, hence not due to recombination. Both of these exceptions were probably phenotypic variants of the expected tailless class.

TABLE 3

*Results of crosses between tailless animals of Line* **29** *by tailless from Line A.*

| MOTHER | FATHER | PROGENY | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | NORMAL TAIL ($t^0t^1$) | | | | TAILLESS ($Tt^0$; $Tt^1$) | | | | |
| | | ♀ | ? | ♂ | Total | ♀ | ? | ♂ | Total | n |
| Line A ($Tt^0$) | Line 29 ($Tt^1$) | 142 | 4 | 93 | **239** | 266 | 5 | 222 | **493*** | **732** |
| Line 29 ($Tt^1$) | Line A ($Tt^0$) | 68 | 4 | 48 | **120‡** | 176 | | 177 | **353†** | **473** |
| | Totals | **210** | **8** | **141** | **359** | **442** | **5** | **399** | **846** | **1205** |
| Calculated 1:2 ratio | | | | | **401.6** | | | | **803.4** | |
| Deviation | | | | | $-42.6 \pm 16.3$ | | | | | |

* Does not include one animal with stump tail which died before it could be tested.

† Does not include two short-tailed animals, one of which was tested and shown to be $Tt^0$—that is, a phenotypic variant of the tailless type.

‡ Including one animal classed at birth as having tail $\frac{1}{3}$ of normal length; when tested did not transmit $T$, hence was probably phenotypic variant.

The third exception was noted at birth as having a tail slightly truncated at the tip and was suspected of being an accidental variant of the normal class. This proved to be correct, since when tested by normal mates it produced 17 normal young and thus did not transmit $T$. None of these exceptions can be regarded as recombinations.

When calculating the ratio in which the two classes of offspring in table 3 should appear, additional assumptions must be made concerning (1) the ratio of $T$ and $t^0$ or $T$ and $t^1$ gametes from tailless males, (2) the viability of the $t^1t^0$ class. If we assume that both of these are normal, then the ratio of tailless to normal offspring should be as $2/3:1/3$ (compare scheme in fig. 1). The ratio. actually found (846:359) showed a small but significant deficiency of normals. Moreover, among the normals there were observed at birth 210 ♀ ♀ :141 ♂ ♂. The most reasonable assumption was that some of the normal-tailed zygotes die before birth and that this mortality falls most heavily on the males.

A very marked deficiency of normal-tailed animals at birth was found when two particular Line 29 tailless males (brothers) and their progeny were tested by Line A tailless females. The data from these tests are not included in table 3. One of the males produced 49 tailless only; the other produced 26 tailless only. Both produced only tailless when mated with females of their own line, so the exceptional result was not due to misclassification of the parents. Sons and daughters of these exceptional animals (from matings with Line 29 tailless) were tested by Line A tailless and repeated the exceptional behavior of their parents, giving 117 tailless (66 ♀ 51 ♂) and only three normal-tailed offspring, all females. We assumed that the very great deficiency of normal-tailed offspring in these crosses was due to the death of many $t^0t^1$ zygotes before birth, possibly because other genetic factors in the particular Line 29 family adversely affected the development of the weaker $t^0t^1$ type.

The most direct test of such an hypothesis is to look for dead or abnormal embryos at various stages before birth. We therefore examined embryos arising from the cross of Line A tailless by Line 29 tailless and its reciprocal at about the middle of the gestation period (10–15 days after fertilization). Three classes of zygotes are to be expected: Brachy homozygotes, *TT*, recognized by their typically abnormal structure (CHESLEY 1935), tailless, $Tt^0$ and $Tt^1$, and normal-tailed, $t^0t^1$. These would normally be expected in a 1:2:1 ratio, but due to the abnormal segregation ratios of $t^0$ and $t^1$ from males, departures from expectation will occur.

TABLE 4

*Embryos found by dissection of tailless females pregnant by tailless males of the opposite line (A×29; 29×A).*

| FEMALE | PREGNANT BY | DAYS AFTER COPULATION | EMBRYOS CLASSIFIED | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | TAILLESS | *TT* | NORMAL TAIL | SMALL | ABNORMAL | RESORBED | TOTAL |
| 29 tailless | A tailless | 10 | 16 | 5 | — | 5 | 4 | 1 | 31 |
| A tailless | 29 tailless | 11 | 13 | — | 1 | 2 | 2 | — | 18 |
| A tailless | 29 tailless | 12 | 8 | — | 4 | 1 | 1 | 5 | 19 |
| A tailless | 29 tailless | 15 | 3 | — | — | — | — | 2 | 5 |
| A tailless | 29 tailless* | 10 | 19 | 7 | — | 1 | 11 | 3 | 41 |
| A tailless | 29 tailless* | 11 | 5 | 1 | — | — | — | 3 | 9 |
| A tailless | 29 tailless* | 12 | 6 | 2 | — | — | 3 | 6 | 17 |
| | | | | | | | | | 140 |

* Two males which had produced no living normal-tailed young at birth.

The results are shown in table 4. In addition to the three classes expected, three other types of embryos were found. These are referred to in the table as "small," "abnormal," and "resorbed." The first were much smaller than the embryos in the tailless class and were also physiologically much younger; three of the nine had developed far enough so that their tails could be diag-

nosed as normal, while six of them were too young to permit this diagnosis. The "abnormals" were not only smaller and younger than their tailless littermates but showed definite new abnormalities: microcephaly, microphthalmia, and even anencephaly. Only three were enough developed to permit tail diagnosis; these had normal tails. The dead and resorbed embryos could not be classified. One died in the decidua stage probably from accidental causes; the others died after formation of the chorion.

In order to exclude the possibility that the microcephalic and microphthalmic embryos might represent *TT* embryos, modified as a result of outcrossing the stocks, three test matings were made of Brachy ($T+$) females with one of the Line 29 tailless males ($Tt^1$) which had given microcephalics in crosses with $Tt^0$ females. The females were dissected after 11 and 12 days of pregnancy. Out of 21 embryos found, 20 were clearly normal-tailed, Brachy or tailless, while one dead embryo seemed abnormal and possibly microcephalic, although it was too necrotic to permit diagnosis. On the other hand, in two matings of $+t^0$ female by $+t^1$ male, where no abnormal genotype except $t^0t^1$ is expected, two definitely abnormal microcephalic embryos were found out of a total of 22, as well as two physiologically younger embryos and four others which had died after formation of a chorion. From crosses of Line 29 tailless by Line A tailless occasional small normal-tailed animals are found at birth, and one of these showed a marked microphthalmia.

The fact that no embryos like those classed as abnormals above, nor embryos retarded to such an extent as those classed as "small," had been found in any of the large number of litters from other experiments dissected in this laboratory seems to justify their inclusion in the $t^0t^1$ genotype. All that lived long enough showed normal tails, and in the cross made only $t^0t^1$ has this phenotype.

These statements apply both to the progeny from "regular" tailless males and to those from the exceptional males which produced no living normal-tailed young at birth. In the case of the latter, however, the proportion of abnormal embryos was high, all fourteen of them were microcephalic, and of these, two were enough developed to be diagnosed as normal-tailed. Four control litters from matings of these exceptional males by females of their own line (29) yielded no abnormal embryos in a total of 25. The microcephalic embryos are considered to represent the $t^0t^1$ genotype which in the exceptional family suffers a much higher mortality and is nearly always abnormal.

The answer to our question is clear: normal-tailed $t^0t^1$ embryos suffer a heavier mortality and show certain abnormalities by which many of them may be identified. The factors which cause only certain of the $t^0t^1$ embryos to become microcephalic or microphthalmic abnormals are unknown, but indications have been found of the presence of factors other than those at the *T* locus which tend to reduce the viability of the $t^0t^1$ zygote.

On the basis of these facts it is now possible to explain the peculiar circumstance that, in spite of the fact that $t^0$ and $t^1$ sperm are transmitted with abnormally high frequency by $Tt^0$ and $Tt^1$ males, the zygotes ($t^0t^1$) arising from such sperm in crosses between $Tt^0$ and $Tt^1$ appear at birth in abnormally low

frequency. The deficiency of $t^0t^1$ is due to prenatal mortality brought about jointly by the action of $t^0$ and $t^1$ supplemented by other factors which have not become constant in our stocks. Variations in the ratios of $t^0$ and $t^1$ sperms from $Tt^0$ and $Tt^1$ males have been shown in new experiments (unpublished) to be independent of the variations causing prenatal mortality of $t^0t^1$. Consequently the varied proportions of normal-tailed offspring appearing from crosses between different balanced tailless lines are due to variations in viability superimposed upon variations in sperm frequency.

### TEST OF RECOMBINATION IN $t^0t^1$ FEMALES

We may now utilize the compound type $t^0t^1$ for a test of recombination between $t^0$ and $t^1$. Males $t^0t^1$ are entirely sterile (DUNN 1937; BRYSON 1942) and cannot be used. The best test is to mate females $t^0t^1$ (from crosses between tailless A and tailless 29, and from crosses of $+t^0 \times Tt^1$ and of $+t^1 \times Tt^0$) with Brachy males ($T+$). If no recombination occurs, the progeny from $t^0t^1 \times T+$ will consist of only two classes: tailless ($Tt^0$, $Tt^1$) and normal tail ($+t^0$, $+t^1$); recombination gametes $++$ would lead to production of normal and of Brachy offspring, while recombination gametes $t^0t^1$ would probably lead to the same phenotypes as $t^0$ or $t^1$ gametes. In practice then only the exceptional Brachy offspring could be detected, and these should represent half of the $++$ recombination gametes produced. Although the test is only 50 percent efficient, it is otherwise reliable.

TABLE 5

*Results of matings of normal-tailed females $t^0t^1$ [from crosses of Line 29 $\times$ Line A] by Brachy males ($T+$).*

| DERIVATION OF $t^0t^1$ | $t^0t^1$ FEMALES | OFFSPRING BY BRACHY MALE | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | NORMAL TAIL | | | | SHORT TAIL | TAILLESS | | | |
| | | ♀ | ? | ♂ | TOTAL | | ♀ | ? | ♂ | TOTAL |
| ♀ $Tt^0 \times$ ♂ $Tt^1$ | 45 | 279 | 7 | 261 | **547** | 2! | 268 | 9 | 257 | **534** |
| ♀ $+t^0 \times$ ♂ $Tt^1$ | 5 | 38 | 3 | 28 | **69** | | 14 | 4 | 23 | **41** |
| ♀ $Tt^1 \times$ ♂ $Tt^0$ | 24 | 158 | 9 | 145 | **312** | | 133 | 10 | 126 | **269** |
| ♀ $+t^1 \times$ ♂ $Tt^0$ | 4 | 14 | | 26 | **40** | | 14 | | 18 | **32** |
| Totals | 78 | 489 | 19 | 460 | **968** | 2 | 429 | 23 | 424 | **876** |

The results of the tests are shown in table 5. In general, each of the 78 $t^0t^1$ females from whatever source produced two classes of young when bred to Brachy males—normal-tailed and tailless in about equal numbers. There is a small deficiency of tailless progeny, probably due to a somewhat lower viability of this type. Among the 1846 offspring examined there were two exceptional individuals. One of these had a short tail one-third of the normal length and was classed as a Brachy. This animal proved to be a phenotypic variant of the tailless type, since when crossed with Brachy it produced normal, Brachy and tailless progeny; crossed with tailless Line 29 it gave tail-

less only (5); while when tested by a Line A male it gave tailless and normal only (4:2). This behavior is consistent with the assumption that the genotype of the exception was $Tt^1$, hence its origin did not involve recombination.

The other exception had an even longer tail, about one-half the normal length and was also classed as Brachy. However, when tested by Line 29 tailless it gave only tailless progeny (4), and by Line A tailless it gave tailless and normal (7:3) and no Brachys. Its genotype was thus $Tt^1$, and it, too, was a phenotypic variant of the tailless type, albeit a rather extreme one. Both of these cases occurred after the tailless balanced lines had been outcrossed and probably are due to segregation of modifiers as discussed above.

We may thus conclude that no evidence of recombination was observed in 1846 tested gametes, although since only half of the ++ recombinations could be detected, the ratio is better expressed as 0/923.

### SUMMARY OF RECOMBINATION DATA

The data from the several matings in which recombination could be detected are summarized in table 6. The 6775 negative observations should be reduced by about 50 percent, since in most cases only half of the possible recombinants could be detected. Even this figure justifies the conclusion that recombination, if it occurs at all, is so rare as not be detectable in any ordinary sample. In all cases, progeny tests of the rare individuals which phenotypically resembled recombinants showed that they were, in fact, phenotypic variants of the non-crossover type.

TABLE 6

*Summary of matings in which recombination among T, $t^0$ and $t^1$ could be detected.*

| PARENTS | TOTAL CLASSIFIED AS NON-RECOMBINANTS | REFERENCE |
|---|---|---|
| Tailless balanced (intra-line) | 2976 | Table 1 |
| $F_1$'s tested from tailless×normal | 411 | Table 2 |
| Tailless×tailless (inter-line) | 1205 | Table 3 |
| Tailless×tailless (inter-line) | 195 | page 34 |
| Tailless×tailless (19×A; 29×19) | 142 | DUNN 1939 |
| $t^0t^1$×Brachy (+$T$) | 1846 | Table 6 |
| Total | 6775 | |

### DISCUSSION

Since no recombination among the three factors concerned has been found, we are free to regard them either as unilocal or as occupying different loci among which crossing over is prevented. If we choose the second alternative, the simplest assumption would be that the dominant factor $T$, which is common to all the balanced lines, is itself the factor which prevents crossing over. This, however, cannot be the case, since $T$ does show crossing over with two adjacent loci *Ki* and *Fu* (DUNN and CASPARI 1942). It would then be necessary to assume that both of the other factors, $t^0$ and $t^1$, represent chromosome re-

arrangements or otherwise prevent crossing over. There is no evidence, like that above, to disprove this assumption. Neither $t^0$ nor $t^1$ has yet shown any recombination with *Ki* or *Fu*, although these tests are not yet completed. No other loci in the *T* chromosome are available for tests with $t^0$ and $t^1$. If these mutations are rearrangements (for example, inversions), they probably represent different aberrations, since although each is lethal when homozygous, the compound $t^0t^1$ may be viable. The gross morphological effect of each is recessive, although this cannot be used as an argument against the rearrangement hypothesis, since each mutation has other dominant effects on segregation (DUNN and GLUECKSOHN-SCHOENHEIMER 1939) and since many position effects are recessive. One is reminded here of the case described by DUBININ and SIDOROFF (1934) in which each of two different wild type alleles in *Drosophila melanogaster* was so modified by translocation as to acquire different properties, although the compound of the two modified alleles was normal. If $t^0$ and $t^1$ are wild type alleles and owe their properties to their position, then because of their similar properties we should have to regard them as different alterations of the same wild type allele. In the absence of any evidence of chromosomal change, this hypothesis cannot be distinguished from that which regards them as different alterations of the same locus *in situ*.

However, if we assume that *T*, $t^0$, and $t^1$ are not unilocal, we must explain the peculiar phenotypic interactions among these factors which in many ways resemble those to be expected of alleles. Thus $t^0$ and $t^1$ each interact with *T* to produce taillessness, although they do not have this effect in combination with the similar and nearby but non-allelic mutations *Ki* and *Fu* (DUNN and CASPARI 1942) nor with any other of the several tail mutations with which they have been tested. The mutations $t^0$ and $t^1$ apparently have just the same interaction effect with *T* and are alike in the otherwise unique property of altering segregation ratios and producing "clusters" of offspring of one genotype. The two mutations answer all the criteria of allelism except that the compound $t^0t^1$ has a normal tail. That the two mutations do interact both morphologically and physiologically is shown by the appearance of microcephaly and microphthalmia and of complete male sterility in the compound. The above facts coupled with the absence of recombinations between $t^0$ and $t^1$ strongly suggest that they are alleles. The present evidence also makes it reasonable to assume that *T* is an allele of $t^0$ and $t^1$, although a final conclusion cannot be drawn now.

The evidence seems further to justify certain speculations regarding mutation and the relations of alleles. It has often been assumed that multiple allelic series arise by repeated mutations of the same locus which differ in the degree of alteration induced in the locus. These series often show clearly the quantitative gradation of which the alleles of white in Drosophila or albinism in rodents have long been familiar examples. This has led to the assumption that some single process has been changed quantitatively by successive reductions from its normal level. This is without doubt a reasonable view with respect to effects of the same kind, as for example pigmentary effects. But it is also evident that not all effects of changes at the same locus are of the same kind. In the case

described in this paper effects of different kinds accompany genetically unitary alterations like $t^0$ or $t^1$. The structure of the tail and axial skeleton is one type of effect; the behavior of the genetic factors in spermatogenesis is another, to which the partial sterility effect is probably related (BRYSON 1942); while the early lethal effect may be another. These different effects while related genetically show a certain degree of independence in development. The most reasonable guess here would seem to be that each of the three mutations alters some part of one process underlying the organization of the axial system. The same mutation may or may not alter another process underlying viability, since in this respect $t^0$, $t^1$, and $T$ act as though they affected different processes. Here we must note, however, that under certain conditions $t^0$ and $t^1$ do interact to lower the viability of the compound, although this may occur because development has been rendered unstable by reason of their effect on a common organization process. The effects of $t^0$ and $t^1$ on spermatogenesis are clearly similar and show cumulative interaction in the sterility of the $t^0t^1$ males; yet $T$ does not show this effect at all nor does it enhance this effect when combined with $t^0$ or $t^1$.

To point out differences among the pleiotropic effects of alterations in one genetic unit, however, may lead to false conclusions, since it is always possible that a complete study of early development might reveal a common source from which diverse effects arise. Pleiotropism, as a descriptive term, merely indicates that several characters are modified by the same mutation. For this reason GRÜNEBERG'S (1938) classification of false and true pleiotropism, the latter restricted to those cases in which the same gene has different primary effects (as assumed in the present case), seems superfluous, since so sharp a distinction could be based only on a knowledge of early developmental events which we do not have and since, a priori, other alternative views are possible. At present it seems wiser to restrict our hypotheses concerning the mechanism of the developmental effect to those which are compatible with diversity in the types of mutation at the same locus even though similarity is the rule; and conversely our conception of mutation should be flexible enough to permit such early diversity in the processes affected by one locus. It seems very doubtful, however, if evidence from one set of facts (developmental or phenotypic affect) can be used to provide positive proof of one view concerning the other—that is, the mode of mutation.

## SUMMARY

Three lethal mutations with similar morphological effects in the mouse, $T$, $t^0$ and $t^1$, were studied in various combinations ($Tt^0$, $Tt^1$, $t^0t^1$) in order to determine whether or not recombination among them occurs. No recombination was found in 6775 test animals.

It is assumed that $t^0$ and $t^1$ are alleles having similar pleiotropic effects on skeleton (taillessness), segregation, and spermatogenesis, with effects on viability which are at least partially different. The compound $t^0t^1$ may be viable or, in combination with other factors, it may lead to typical abnormalities (microcephaly, microphthalmia, and early death).

The factor *T* shows no crossing over with $t^0$ or $t^1$ and is probably an allele, although it does not share the effects of $t^0$ and $t^1$ on spermatogenesis and differs in its lethal effect, since $Tt^0$ and $Tt^1$ are viable.

It is suggested that mutations at the same locus may affect different processes, as well as different parts of the same process.

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# GENETIC INTERRELATIONSHIPS OF DERMATOGLYPHICS AND FUNCTIONAL HANDEDNESS[1]

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INVESTIGATORS of the relationships of dermatoglyphics and functional handedness are in general agreement in finding slight but nevertheless real differences between the dermatoglyphics of right-handed and left-handed populations (BETTMANN 1932; BONNEVIE 1933; CUMMINS, LECHE, and MCCLURE 1931; LECHE 1933; NEWMAN 1934; KEITH'S unpublished data; CUMMINS 1940; CROMWELL and RIFE in press). Left-handers are characterized by slightly less bimanual asymmetry than are right-handers, rather than by outstanding differences in total pattern frequencies. The lowering of bimanual differences in left-handers is especially noticeable in females and is apparent in both palms and finger-tips.

The investigation herewith reported is concerned with the ascertainment of possible genetic interrelationships of pattern development in palm and finger-tip areas with each other and with functional handedness. Assortative mating, pleiotropy, and linkage may each result in certain types of association between biological traits. Obviously, handedness and dermatoglyphics are seldom, if ever, used as a basis for the selection of mates in man, so we need not consider assortative mating further.

Pleiotropic genes result in correlations in the general population between the affected traits. As mentioned previously, several investigators have obtained such data for palmar and finger dermatoglyphics with handedness. WAITE (1915) made an exhaustive analysis of the finger prints of a population of adult males and found that positive correlations exist between pattern frequencies of different fingers. WENNIGER (1935) observed an apparent negative correlation between the occurrence of patterns in thenar and hypothenar areas of palms. KEITH (1924) and BETTMANN (1931) have made similar observations. CUMMINS in a communication to the writer previous to this investigation states his opinion to the effect that pattern occurrences in the thenar/first, second, third, and fourth inter-digital areas are positively correlated with each other and with transverseness of ridges, and also that there is a negative correlation between patterns in hypothenar and thenar[2] areas. No literature has been found dealing with correlations between patterns on palms and on finger-tips.

Linked genes result in inter-trait correlations within sibships, but not within the general population. Thus investigations dealing with the dermatoglyphics of right-handers as compared with left-handers selected from the general population tell us nothing concerning possible linkage relationships. RIFE (1941) obtained evidence for autosomal linkage of genes responsible for pattern

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[2] The term thenar as used in this paper includes both thenar and first interdigital areas.

D in the fourth interdigital area with those responsible for functional handedness. These data were obtained from 24 sibships. No other literature on linkage relationships between handedness and dermatoglyphics or between different dermatoglyphic features is available.

The data included in this report were obtained from 34 sibships, comprising a total of 181 individuals and 548 paired sibs. All the individuals were white, largely descended from northern European stock. Handedness was determined on the basis of testimony and the performance of several unimanual operations, including writing, throwing, sewing, shooting marbles and use of knife and spoon. Those performing one or more of these operations with the left hand were classed as left-handed, those performing all with the right hand were classed as right-handed.

In our analysis of the dermatoglyphics both vestigial and true patterns are classed as patterns. Only loops are included in the third interdigital area, and only D patterns in the fourth interdigital area. Finger-tips are classified as to presence or absence of whorls. Data are included for pleiotropy between the palmar areas with each other and with whorl frequencies on finger-tips. Linkage data are included for each of the palmar areas and whorls on finger-tips with handedness, as well as for the palmar areas with each other and with whorl frequencies on finger-tips.

## PLEIOTROPY

Tests for association between traits were made with $2 \times 2$ tables. When significant relationships were obtained, correlation coefficients were determined by the use of Thurstone's computing diagrams (THURSTONE et al. 1933). Table 1 shows the results of tests for association between patterns on the five palmar areas within single hands. Table 2 shows the results of tests for similar relations between the hands of individuals, and table 3 shows the results of tests for association between frequencies of whorls on finger-tips with pattern occurrence on each of the palmar areas. If the individual has patterns on one or both hands, he is included as patterned. An individual may have from none to ten whorls on finger-tips. In table 3 association between whorl frequency and palm patterns is determined by comparing the frequencies of whorls within palm patterned versus patternless individuals. This type of comparison could not be converted to an actual correlation coefficient by Thurstone diagrams. Whether a loop occurs in the third or fourth interdigital area depends to some extent upon the transverseness of the palmar ridges, third interdigital patterns being more frequently found associated with the distinctly transverse ridges. Thus association with patterns in the third interdigital area is in reality an indication of association with transverseness of ridges.

Note (table 1) that within single hands a negative correlation exists between patterns in hypothenar and thenar areas, in agreement with the observations of other investigators. Furthermore, positive correlations exist between each of the other four palmar areas, the highest correlations generally being between adjacent areas. The high correlation between areas III and IV and also II and III is indicative of a positive correlation between transverseness of ridges and

the occurrence of pattern D in the interdigital areas. This is in complete harmony with the observations of CUMMINS.

TABLE 1

*The frequencies of different combinations of patterned and patternless areas in single palms. $\chi^2$ values are underlined when significant.*

| | PATTERNS ON BOTH AREAS | PATTERN ON FIRST AREA, ABSENT ON SECOND | PATTERNS ABSENT ON FIRST PRESENT ON SECOND | PATTERN ABSENT ON BOTH AREAS | $\chi^2$ | r |
|---|---|---|---|---|---|---|
| *Hypothenar and thenar | 8 | 133 | 37 | 184 | 8.70 | −.40 |
| *Hypothenar and interdigital II | 9 | 132 | 14 | 207 | 0.94 | |
| Hypothenar and interdigital III | 53 | 88 | 88 | 133 | 0.18 | |
| Hypothenar and interdigital IV | 37 | 104 | 69 | 152 | 1.03 | |
| *Thenar and interdigital II | 7 | 38 | 16 | 301 | 6.54 | +.38 |
| Thenar and intergital III | 21 | 24 | 120 | 197 | 1.28 | |
| Thenar and interdigital IV | 17 | 28 | 89 | 228 | 1.79 | |
| *Interdigitals II and III | 16 | 7 | 125 | 214 | 10.65 | +.45 |
| *Interdigitals II and IV | 17 | 7 | 89 | 250 | 21.38 | +.55 |
| Interdigitals III and IV | 67 | 74 | 39 | 182 | 37.11 | +.57 |

* YATE'S (1931) correction used in the calculation of $\chi^2$.

TABLE 2

*The frequencies of different combinations of patterned and patternless palmar areas, resulting from comparisons of right with left and left with right palms of individuals.*

| | PATTERNS ON BOTH AREAS | PATTERNS ON FIRST AREA, ABSENT ON SECOND | PATTERN ON SECOND, ABSENT ON FIRST | PATTERN ABSENT ON BOTH AREAS | $\chi^2$ | r |
|---|---|---|---|---|---|---|
| Hypothenar and thenar | 13 | 128 | 32 | 189 | 2.18 | |
| Hypothenar and interdigital II | 12 | 129 | 11 | 210 | 0.46 | |
| Hypothenar and interdigital III | 58 | 83 | 83 | 138 | 0.46 | |
| Hypothenar and interdigital IV | 35 | 106 | 71 | 150 | 2.21 | |
| *Thenar and interdigital II | 6 | 39 | 17 | 300 | 2.97 | |
| Thenar and interdigital III | 25 | 20 | 116 | 201 | 5.96 | +.20 |
| Thenar and interdigital IV | 15 | 30 | 91 | 226 | 0.40 | |
| *Interdigital IV and III | 17 | 6 | 124 | 215 | 13.86 | +.48 |
| *Interdigital II and IV | 15 | 8 | 91 | 248 | 16.88 | +.47 |
| Interdigital III and IV | 62 | 79 | 44 | 177 | 15.25 | +.43 |

* YATE'S (1931) correction used in the calculation of $\chi^2$.

When both hands of individuals are compared, similar but less marked relationships between palmar areas are found (table 2). Four show significant correlations, and of these the comparatively low correlation between thenar and third interdigital areas is the only one to show an association which was not manifested within single hands. Significant associations are shown between the occurrence of patterns in the second, third, and fourth interdigital areas, and the frequency of whorls on finger-tips. Since no other data showing relationships between whorls and patterns in these areas are on record, the writer made similar comparisons with the prints of the Cromwell series (CROMWELL and RIFE in press) and found the same type of association. No other significant correlations between whorl frequencies and palm patterns are indicated.

TABLE 3

*The frequencies of whorls on finger-tips of individuals having one or more patterns in the palmar areas, as compared with those lacking patterns.*

| | WITH PATTERNS | | WITHOUT PATTERNS | | |
|---|---|---|---|---|---|
| | FINGER-TIPS WITH WHORLS | FINGER-TIPS WITHOUT WHORLS | FINGER-TIPS WITH WHORLS | FINGER-TIPS WITHOUT WHORLS | $\chi^2$ |
| Hypothenar | 668 | 212 | 687 | 243 | 0.99 |
| Thenar | 232 | 78 | 1123 | 377 | 0.00 |
| Interdigital II | 108 | 62 | 1247 | 393 | 12.86 |
| Interdigital III | 803 | 287 | 412 | 108 | 5.92 |
| Interdigital IV | 526 | 224 | 829 | 231 | 15.21 |

## LINKAGE

Tests for linkage were made by the paired sib technique of PENROSE (1935). In regard to hypothenar, thenar/first, second, and fourth interdigital areas, individuals were classed in two categories in respect to each area, those having a pattern on one or both hands and those lacking a pattern on both hands. Tests for associations within paired sibs pattern frequencies in these areas with each other and with handedness were made in $2 \times 2$ tables (table 4). Sib pairs were grouped into four classes—those alike in both traits, unlike in both, and the two combinations of alike in one and unlike in the other. If two traits are linked, pairs alike in both traits and unlike in both should exceed pairs alike in one trait and unlike in the other.

Both whorl frequency and the direction of Line C are quantitative or graded characters. The graded character method of PENROSE (1938) was employed in linkage tests involving these traits. In this method, $\phi$ indicates the degree of linkage, and a $\phi$ value of greater than twice its standard error is considered to be significant. The effect of pleiotropy is removed by subtracting the correlation squared from $\phi$ (see table 5).

Individuals were placed in the following four grades in respect to whorl frequencies: 0, 1–3, 4–6, 7–10. They were classed in three grades in respect to Line C—those having loops formed in the third interdigital areas of both

TABLE 4

*Paired sib combinations of traits tested for linkage with 2×2 tables.*

| | ALIKE IN BOTH TRAITS | ALIKE IN FIRST, UNLIKE IN SECOND | UNLIKE IN FIRST, LIKE IN SECOND | UNLIKE IN BOTH | $\chi^2$ |
|---|---|---|---|---|---|
| Hypothenar and handedness | 196 | 143 | 112 | 97 | 0.94 |
| Thenar and handedness | 259 | 80 | 168 | 41 | 1.21 |
| Interdigital II and handedness | 295 | 44 | 186 | 23 | 0.47 |
| Interdigital IV and handedness | 243 | 101 | 118 | 91 | 11.47 |
| Hypothenar and thenar | 248 | 181 | 64 | 55 | 0.62 |
| Hypothenar and digital II | 270 | 213 | 36 | 29 | 0.007 |
| Hypothenar and interdigital IV | 212 | 151 | 104 | 83 | 0.38 |
| Thenar and interdigital II | 381 | 102 | 43 | 20 | 3.80 |
| Thenar and interdigital IV | 280 | 74 | 168 | 40 | 0.23 |
| Interdigitals II and IV | 292 | 168 | 44 | 21 | 4.37 |

hands, those having a loop in the third interdigital of one hand and in the fourth of the other, and those having loops in the fourth interdigital areas of both hands. Since Line C is abortive or missing in many palms, the number of pairs used in comparison involving Line C is considerably less than in the others.

TABLE 5

*Values obtained in linkage tests involving whorls and Line C, by graded character method. Also checks on other traits indicating linkage in 2×2 tables.*

| | $\phi$ | S.E. | $\frac{\phi}{\text{S.E.}}$ | r | $\frac{\phi - r^2}{\text{S.E.}}$ |
|---|---|---|---|---|---|
| Whorls and hypothenar | +.05 | +.3 | +0.16 | | |
| Whorls and Line C | +.37 | +.21 | +1.76 | | |
| Whorls and thenar | +.06 | +.2 | −0.30 | | |
| Whorls and interdigital II | +.55 | +.21 | +2.60 | | |
| Whorls and interdigital IV | +.06 | +.13 | +0.04 | | |
| Whorls and handedness | +.42 | +.13 | +3.20 | | |
| Line C and interdigital IV | +.59 | +.14 | | +.57 | +1.89 |
| Line C and hypothenar | +.02 | +.16 | +0.12 | | |
| Line C and thenar | +.22 | +.23 | +0.93 | | |
| Line C and interdigital | +.05 | +.26 | +0.19 | | |
| Line C and handedness | −.08 | +.11 | −0.07 | | |
| Interdigital IV and handedness | +.36 | +.10 | +3.60 | | |
| Interdigital IV and interdigital II | +.03 | +.21 | +0.14 | | |
| Cumulative pattern and whorl frequencies with handedness | +.57 | +.11 | +5.18 | | |

The cumulative frequency values of whorls and patterns are estimated on the basis of patterns in all palmar areas, except the hypothenar, and on finger tips of both hands. Whorl frequencies are grouped in the same four categories as for the other comparisons, while patterns on each of the four areas under

consideration are given a value of 1. Thus the cumulative patterns and whorl values of individuals range from 0 to 7. If an individual has patterns on each of these areas on one or both hands, and whorls on seven or more finger-tips, he is placed in grade 7; if fewer, he is classified accordingly. Hypothenar patterns are omitted because of their negative correlations with thenar patterns:

An examination of tables 4 and 5 shows associations between the following. handedness with pattern D in the fourth interdigital area, handedness with whorl frequencies, patterns in the second interdigital area with whorl frequencies, and pattern frequencies in second and fourth interdigital areas, and between the cumulative frequencies of whorls on finger tips and patterns on palms with handedness. The highly significant indications of linkage between handedness and pattern D are in accord with the earlier findings of RIFE (1941). As a further check, these same areas were tested for linkage by the graded character method, with a resultant highly significant relationship (table 5). The relationship between whorl frequency and handedness is also highly significant, although less than that between pattern D and handedness. The indicated relationship between whorls and interdigital area II is significant but not highly so. In view of the fact that patterns occur only rarely in the second interdigital area and that they occurred in only five of the 34 sibships included in this investigation, the test results should probably not be considered as conclusive evidence of linkage between these traits. The linkage relationships between areas II and IV were also tested by the graded character method and, as shown in table 5, no linkage is indicated.

## DISCUSSION

Pattern occurrence in each of the palmar areas, transversness of palmar ridges, and whorl frequencies on finger-tips all show one or more inter-correlations. Highest correlations are usually obtained within palms when single rather than paired palms are compared. Associations characteristic of autosomal linkage are shown between handedness and whorl frequencies on finger-tips, handedness and pattern D in the fourth interdigital area, and cumulative pattern and whorl frequencies on palms and finger-tips with handedness.

Questions may arise as to why we do not also find evidence of linkage between patterns in the second interdigital area and the direction of Line C with handedness, since both show positive correlations with pattern D in the fourth interdigital area. Patterns occur only rarely in the second interdigital area, and in the absence of complete correlation between patterns in the two areas, it is not surprising that evidence for linkage of patterns in the second interdigital area with handedness is lacking. The correlation between patterns in the third and fourth interdigital areas actually is largely a one way correlation. A loop is usually formed in the third interdigital area when Line C swings in a radial direction, whereas a loop is usually formed in the fourth interdigital area when it swings in an ulnar direction. In the presence of pattern D in the fourth interdigital area, Line C usually forms a loop in the third interdigital area, very seldom in the fourth. In the absence of pattern D, however, loops occur with not greatly different frequencies in the third and

fourth interdigital areas. Due to abortive conditions of Line C or its complete absence in one or both palms of several individuals, only 273 pairs were available for comparisons. In view of these circumstances, one would not necessarily expect indications of linkage between Line C and handedness to appear.

While both whorl frequencies on finger tips and patterns in the fourth interdigital area show evidence of linkage with handedness, they show no indication of linkage with each other. This need cause no concern, since genes conditioning each of the traits may conceivably be located on a single chromosome, the gene affecting handedness being located midway between genes affecting whorls and patterns. The techniques employed for testing linkage are not precise enough for the detection of linked factors with high crossover percentages. It is also possible that two independent pairs of factors may affect handedness, one of which is linked with genes affecting whorl frequencies, and the other linked with genes affecting pattern development in the interdigital areas. In view of the association manifested between whorl frequencies and interdigital patterns, however, the most likely possibility is that the genes responsible for the correlation between whorls and interdigital patterns are linked with genes determining handedness. This is borne out by the relations between cumulative finger whorls and palm patterns with handedness. Other sets of genes likely influence the development of different areas independently, thus partially accounting for the lack of complete correlation between them.

Findings from comparative anatomy and human embryology would seem to lend support to this latter view (WHIPPLE 1904; CUMMINS 1929). Pattern areas on palms and finger-tips, as well as on soles and toes, are homologues of the volar or walking pads of mammals. During embryological development these pattern areas are much more elevated than at birth. Although the phylogenetic record is incomplete, there is good reason to believe that the degree of pattern development is positively associated with the degree of elevation of pattern areas and that the evolutionary trend has been toward a reduction in degree of elevation with a corresponding decrease of pattern intensity.

These changes have not progressed uniformly in the various pattern areas or even in right and left hand, as attested by the well established differences in pattern frequencies. Mutations affecting specific areas could account for such differences, while mutations affecting genes having a common effect on area elevation would be responsible for the general evolutionary trend toward reduction in pattern frequencies. Genes having a general effect on the degree of elevation and pattern intensity seem likely to be responsible not only for the observed correlations, but also possibly to be linked with genes determining handedness. Such linkage relationships would be more easily detected in pattern D and finger-tip whorls, because of the comparatively high occurrence of patterns in these areas.

## SUMMARY

Within single hands, negative correlations exist between the occurrence of patterns in hypothenar and thenar areas. Positive intercorrelations exist

between pattern frequencies in thenar and second interdigital areas, between pattern frequencies in the second, third and fourth interdigital areas, and between the transversness of ridges and interdigital pattern frequencies.

Within individuals, positive correlations exist between pattern frequencies in the second and fourth, the second and third, and the third and fourth interdigital areas. Frequencies of whorls on finger-tips are positively associated with pattern frequencies in the second, third, and fourth interdigital areas.

Paired sib comparisons show associations characteristic of autosomal linkage between factors responsible for D patterns in the fourth interdigital area with handedness, for whorl frequencies on finger-tips with handedness, and for combined whorl and pattern frequencies on finger-tips and palms with handedness.

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# STUDIES ON THE INTERACTION OF MUTATIONS AFFECTING THE CHAETAE OF DROSOPHILA MELANOGASTER. II. THE RELATION OF CHARACTER EXPRESSION TO SIZE IN FLIES HOMOZYGOUS FOR POLYCHAETOID, HAIRY, HAIRY WING, AND THE COMBINATIONS OF THESE FACTORS

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THE study of the results of combining various genetic factors affecting a particular embryonic system permits certain inferences concerning the nature of gene action. Favorable material for such a study is supplied by three *Drosophila melanogaster* mutations whose chief detectable effects are to increase the number of chaetae present on the fly. In the first paper of this series (NEEL 1941) it was reported that the phenotype of a combination of any two or all three of these mutations generally represented more than the sum of the effects of these mutations when acting separately. A barrier to an analysis of the developmental reactions revealed by these data was the following circumstance: the eight different genotypes included in the study were in some cases characterized by significantly different mean fly sizes. Since previous work (MACDOWELL 1915, NEEL 1940a) had shown that chaetae number is correlated with fly size, any attempt to analyze the data without first correcting for the size differences between genotypes would be to introduce an initial bias; the data then on hand were not considered adequate to make this correction.

In the present paper the equations for the regression on fly size of each of the five chaetal characteristics studied in the different genotypes are derived where possible. These equations are then utilized in the analysis of two related questions: (1) What do the interaction data, corrected for size differences, reveal concerning the embryonic reactions involved in the formation of supernumerary chaetae? (2) How are the regression coefficients in a genotype *ab* related to the coefficients observed in either *a* or *b*? This latter question is one on which we have at present little information, important though it is to the problem of related increases in the magnitudes of two measurements.

## MATERIALS AND METHODS

The factors utilized in this study were Hairy wing (*Hw*, 1–0.0+), hairy (*h*, 3–26.5), polychaetoid (*pyd*, 3–39 ± 2), yellow (*y*, 1–0.0), and sepia (*se*, 3–26.0). The first three factors increase the number of chaetae present. The *y* and *se* genes have no known effects on the chaetae but were used in the crosses as markers of *Hw* and *h*, respectively. After *y Hw*, *se h*, and *pyd* strains had

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been rendered highly isogenic with a long inbred Oregon wild type line through repeated crosses to, and extractions from, this line, the possible combinations of the three factors affecting the chaetae were synthesized. The study thus includes a total of eight genotypes: $+^{Or}$, *y Hw*, *se h*, *pyd*, *y Hw; se h*, *y Hw; pyd*, *se h pyd*, and *y Hw; se h pyd*. The considerations dictating the choice of these particular mutations and details concerning the synthesis of the stocks are given in the earlier paper.

Five characteristics pertaining to the chaetae were investigated: number of dorsocentral bristles, number of scutellar bristles, number of scutellar hairs, number of hairs on the right second longitudinal wing vein, and number of teeth in the right sex-comb. For each genotype the relation of these characteristics to mean fly size, as measured by femur length, was derived in the following manner: Flies of the desired genotype were allowed to oviposit on small food chips for eight to 12 hours. At the end of this time the eggs were divided into lots of either 100 or 200, and each lot placed in a 23×95 mm shell vial which contained 10 cc of a yeast suspension absorbed on one sheet of Kleenex, an absorbent paper preparation. The concentration of the yeast suspension in different vials varied between 2.5 and 25 percent. By regulating the number of eggs introduced into the vial and the concentration of the yeast suspension, it was possible to get groups of flies of very different mean sizes. All experiments were conducted at 25.0±0.3°C. To establish the nature of the regression obtaining in any one genotype, groups of 50 male flies raised at a particular food level were investigated as to mean femur length and the mean condition of each of the five chaetal characteristics. The data on each fly were kept separate. The examination of eight or nine such groups raised at different food levels gave a sufficient spread in the mean values of the characteristics to permit a derivation of the regression coefficients, using the method of least squares. To be certain of a considerable distribution in the values of the means, it was customary to select from the group raised at the lowest food level (initial condition: 200 eggs on 10 cc of a 2.5 percent yeast suspension) the 50 smallest males and derive the mean values in this special group.

The duration of the egg-larval-pupal stage is longer when flies are raised at a low food level than when food conditions are more favorable. In an earlier experiment, no relation between the length of egg-larval life and the number of dorsocentral bristles could be detected in *pyd* flies nor in flies containing another bristle mutation, Dichaete (NEEL 1940a). It is assumed, in the absence of any evidence to the contrary, that the same lack of correlation between character expression and the duration of development is true for all chaetae, both in *pyd* and in *Hw* and *h* flies. The differences in the length of the life cycle in different groups of flies, resulting from the technique employed, are thus regarded as having no effect upon the regressions observed.

## RESULTS

### *The regressions in the different genotypes*

The complete results of this procedure are too bulky for publication, but are on file with GENETICS. The exact nature of the regressions of the various chaetal

characteristics on femur length is difficult to determine when one of the variables, femur length, shows such relatively small variations. Thus, in any one case the regression shows good conformity to a straight line no matter whether the data are plotted arithmetically, logarithmically, or semi-logarithmically. Accordingly, the regressions were derived on the assumption of a linear relationship between the expression of the chaetal characteristic and femur length. The regressions, derived by the method of least squares, were of the form $E=ax+b$, where x is the femur length, a the slope of the regression line, b the intercept on the Y-axis, and E consequently the estimated value of the characteristic at the given femur length. Values of a and b are given in table 1. Where a character showed no, or very slight, variation, regression constants could, of course, not be obtained. In a very few instances where the departure from wild type shown by a characteristic in a particular genotype was small, at lower body sizes this characteristic appeared essentially normal. In such cases the regression was derived only over that size range in which appreciable variation occurred. The range in femur length over which any one characteristic was observed to vary sufficiently from wild type to be studied is indicated in table 1. The average number of paired determinations used in the derivation of each of the 33 regressions was 451.5 (9.03 groups). The standard error of estimate of E is also given in the table, as an indication of the dispersal of the points about the line of best fit. The regression equations are plotted in figure 1. The projection of the regression line on the abscissa indicates the range in mean femur length studied, while its projection on the ordinate indicates the approximate range in the chaetal characteristic.

*The effect of modifiers on the expression of the three factors*

The differences between these eight genotypes which are described in table 1 and figure 1 are undoubtedly for the most part referable to the known genetic differences between the strains. However, an unknown portion of these differences may be ascribed to an initial failure to render these lines completely isogenic for all factors except the recognizable mutations under investigation, and to the appearance of different modifying factors in the strains during the eight to ten months that elapsed between the synthesis of the strains and their use in this study.

The *se h* data are pertinent in this connection. When the data on this genotype were first collected, they led to the following regressions (each regression based on 500 paired determinations (10 groups) ): scutellar hairs: $E=0.0631x-7.389$; scutellar bristles: $E=0.00289x+2.673$; wing hairs: $E=0.0377x-8.726$; sex-comb teeth: $E=0.0116x+4.102$. When the values predicted on the basis of these equations were compared with the values reported in the earlier paper (NEEL 1941), a discrepancy was apparent. Accordingly, the *se h* strain was crossed to, and the factors extracted from, the $+^{Or}$ line three additional times. The regressions were again derived and found to be in better agreement with previous results. It is this second set of *se h* data which is summarized in table 1 and figure 1. At a femur length of 525$\mu$ the following differences exist between the values predicted on the basis of the two sets of

TABLE 1

*The regression of the five chaetal characteristics on femur length, in the eight different genotypes. Where the range of variation is small, or where there is only sporadic variation, no regression constants have been calculated. The constant* a *represents slope, and* b *the* Y*-intercept, in an equation of the form* E = ax + b, *where* x *is mean femur length and* E *the estimated value of the characteristic at this femur length. The error appended to the* a *values is the standard error of estimate of* a. *The constant* $s_{y \cdot x}$ *is the standard error of estimate of* E. *The figure in parentheses after the "range of* x*" indicates the number of groups of 50 flies each included in that range.*

| CHARACTER | | GENOTYPE | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | + | *pyd* | *y Hw* | *se h* | *y Hw; pyd* | *y Hw; se h* | *se h pyd* | *y Hw; se h pyd* |
| | Range of x | — | 420.5–549.4 (9) | 456.8–558.6 (9) | — | 414.9–556.2 (10) | 415.0–543.5 (9) | 455.8–529.6 (8) | 418.9–538.4 (9) |
| Dorsocentral bristle | a | — | .0116 ± .00134 | .0156 ± .00206 | — | .0241 ± .00268 | .0168 ± .00198 | .0148 ± .000663 | .0224 ± .00250 |
| | b | — | −.693 | −2.275 | — | −3.364 | −.0251 | −2.511 | −1.412 |
| | $s_{y \cdot x}$ | — | .173 | .206 | — | .374 | .227 | .0500 | .286 |
| | Range of x | — | 420.5–549.4 (9) | 492.3–558.6 (8) | 511.0–553.1 (6) | 414.9–556.2 (10) | 415.0–543.5 (9) | 406.1–529.6 (9) | 418.9–538.4 (9) |
| Scutellar bristle | a | — | .00135 ± .000512 | .000987 ± .00138 | .00264 ± .00222 | .00356 ± .000679 | .0107 ± .00140 | .00237 ± .000645 | .0149 ± .000900 |
| | b | — | 3.482 | 3.580 | 2.684 | 2.695 | −.0129 | 3.155 | −1.476 |
| | $s_{y \cdot x}$ | — | .0660 | .0898 | .0857 | .0948 | .160 | .0769 | .103 |
| | Range of x | — | — | 456.8–558.6 (9) | 418.7–553.1 (9) | 414.9–556.2 (10) | 415.0–543.5 (9) | 406.1–529.6 (9) | 418.9–538.4 (9) |
| Scutellar hairs | a | — | — | .0104 ± .00230 | .0717 ± .00396 | .0145 ± .00187 | .0887 ± .0109 | .0949 ± .00329 | .0832 ± .0102 |
| | b | — | — | −1.529 | −14.854 | −2.829 | −5.357 | −14.275 | 6.615 |
| | $s_{y \cdot x}$ | — | — | .231 | .528 | .261 | 1.251 | .393 | 1.169 |
| | Range of x | — | — | 456.8–558.6 (9) | 418.7–553.1 (9) | 414.9–556.2 (10) | 415.0–543.5* (9) | 406.1–529.6 (9) | 418.9–538.4 (9) |
| Wing hairs | a | — | — | .0147 ± .00376 | .0253 ± .00713 | .0247 ± .00297 | .0640 ± .0129 | .0125 ± .00307 | .0926 ± .00550 |
| | b | — | — | −1.467 | −7.533 | −2.615 | .385 | 1.928 | −5.842 |
| | $s_{y \cdot x}$ | — | — | .377 | .949 | .415 | 1.478 | .366 | .631 |
| | Range of x | 429.7–564.6 (10) | 420.5–549.4 (9) | 456.8–558.6 (9) | 418.7–553.1 (9) | 414.9–556.2 (10) | 415.0–543.5 (9) | 406.1–529.6 (9) | 418.9–538.4 (9) |
| Sex-comb teeth | a | .00861 ± .000828 | .0118 ± .00113 | .0111 ± .00160 | .00905 ± .00138 | .0154 ± .000640 | .0125 ± .000975 | .0122 ± .00116 | .0176 ± .00101 |
| | b | 5.696 | 3.640 | 4.656 | 5.633 | 2.196 | 2.990 | 4.832 | 2.105 |
| | $s_{y \cdot x}$ | .124 | .146 | .160 | .183 | .0894 | .112 | .138 | .116 |

* These values considerably influenced by one aberrant point.

regressions: scutellar hairs, $3.01 \pm 0.86$; scutellar bristles, $0.11 \pm 0.12$; wing hairs, $5.31 \pm 1.27$; and sex-comb teeth, $0.18 \pm 0.33$. In both cases where the differences are significant, the strain which had been crossed the three additional times showed the lower values. These results are most easily explained by assuming the accumulation in the strain of one or more positive modifiers of some of the chaetal characteristics under consideration, with the loss of these

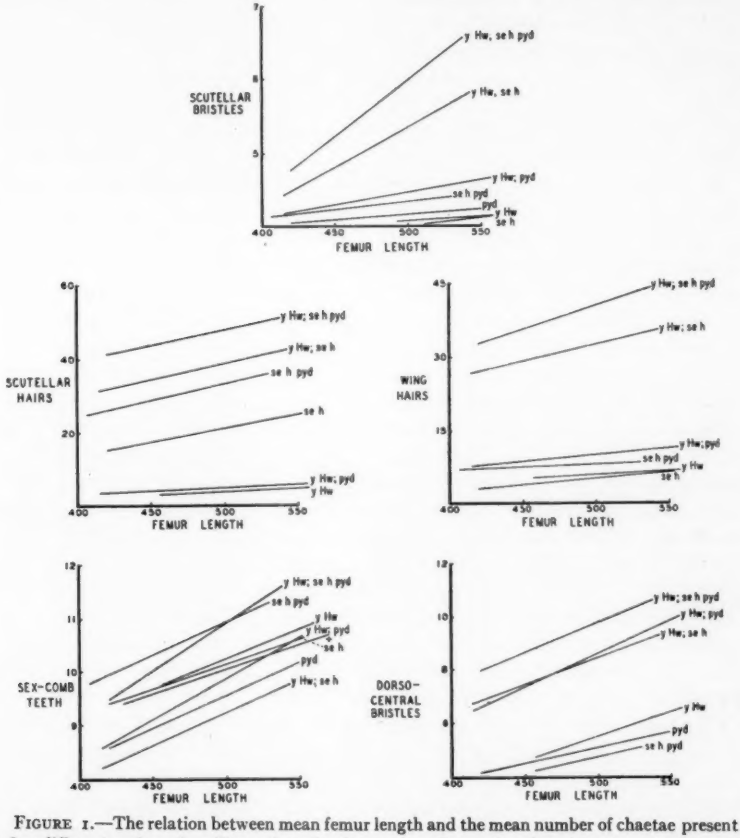


FIGURE 1.—The relation between mean femur length and the mean number of chaetae present in five different regions, in *y Hw, se h, pyd, y Hw; se h, y Hw; pyd, se h pyd, y Hw; se h pyd,* and wild type *Drosophila melanogaster* males. Details in text.

modifiers on outcrossing. The modifiers appear to be rather specific, in that they affect those two of the four characteristics under observation which are phenotypically most similar.

In the *se h* case, the accumulation of modifiers was first detected because of the relatively large effect on the wing hairs. Comparison of the present data with the earlier results suggests that other less striking modifiers may have

accumulated in several strains. HERSH (1930) has recorded a similar rapid appearance of modifiers of a quantitatively variable characteristic in *Drosophila melanogaster*, Bar eye. Moreover, there can be little doubt that various wild type strains, in spite of their anatomical similarities, differ greatly with respect to their complement of minor modifying factors (*cf.* PIPKIN 1942). The interaction of these three factors might therefore appear significantly different if they were studied on another genetic background. It is obvious that under such circumstances one must be cautious in the development of theoretical treatments of the data.

## ANALYSIS OF THE DATA

It is apparent from figure 1 that in the cases of two of the five characteristics studied (number of scutellar hairs, number of hairs on the right second longitudinal wing vein) the relations of the various regressions on femur length are essentially the same. In each case there is evident a family of straight lines in which, within the range of chance variation, at any given value of x over the range in x studied the slope of any single line tends to be directly proportional to the magnitude of its y value. In each case this series of lines can be transformed to a family of lines whose slopes are not significantly different by plotting the ordinate logarithmically. Moreover, the slopes of the wing hair regressions are quite similar to those of the scutellar hair regressions. Such a treatment of a third characteristic, the scutellar bristles, although it lessens the differences between lines with respect to their slopes, still leaves a situation where at a given x value the equations yielding the largest y values have the greatest slopes. But if in this latter case one plots as ordinate the logarithm of the bristle number minus four, one secures a family of parallel lines. The theoretical justification in the subtraction of four lies in the fact that normally three are four scutellar bristles present; the bristle number minus four is thus the number of extra bristles present. There are in the wild type only very sporadic hairs present on the scutellum, and none on the second longitudinal wing vein, so that the values shown for these characteristics refer without correction to the number of extra chaetae. In these three regions, then, the number of extra chaetae is apparently related to the size of the fly in a similar manner.

The situation with respect to the dorsocentral bristles appears to be different. Thus, when the ordinate is taken as the logarithm of the number of extra bristles, the smallest regression coefficient (*y Hw; se h*) is $0.00183 \pm 0.000216$, and the largest (*se h pyd*), $0.00877 \pm 0.000480$. But when the logarithm of the total number of dorsocentrals is plotted against femur length, there is a reasonable approximation to parallelism, with the greatest difference between any two slopes (*y Hw; se h—se h pyd*) being $0.000423 \pm 0.000484$. While it is impossible to say to what extent this situation is due to the operation of chance and accumulated modifiers, the fact, to be discussed below, that the type of interaction seen here differs from that in the preceding three systems suggests that this difference is real and characteristic of the region.

The sex-comb data present a unique picture. The variation in the mean number of teeth in the right sex-comb is so small that the relations of the data

are not materially altered by logarithmic plotting. The seriation with respect to the steepness of the regressions on an arithmetic plot is as follows: $+^{Or}$, *se h*, *y Hw*, *pyd*, *se h pyd*, *y Hw; se h*, *y Hw; pyd*, and *y Hw; se h pyd*, with the latter two separated from the former by quite a gap. Unlike the other systems, this seriation does not appear to be related to the magnitude of the departure from wild type, but rather to the number of mutations present in the strain. For instance, although the regression coefficient for *y Hw; se h* is significantly greater than for $+^{Or}$, flies of the former genotype have significantly fewer teeth in the sex-comb at all sizes studied. The relative steepness of the regression in the two genotypes containing *y Hw* and *pyd* may indicate a type of specific interaction.

As pointed out above, the present data, because of the relatively small range of the variables, can equally well be treated as an expression of a linear relation ($y=ax+b$) or of an exponential function ($y=bx^k$); for simplicity's sake the former treatment was chosen. Since the variations in mean femur length are relatively small (although corresponding to an approximately six- to eightfold variation in body weight), conversion of the femur lengths to their logarithmic equivalents would not alter the relative femur lengths to an important extent. The conversion of the considerably more variable numbers of hairs and bristles, as described above, therefore gave essentially the same picture as would have resulted from treating the data as an expression of the second relation, which in its logarithmic form becomes $\log y=\log b+k \log x$. In view of the large amount of work done on biological relationships of this nature, it is of interest to consider the data from this angle.

Although the equation $y=bx^k$ has been found to describe a variety of cases of correlated increase in two measurements, the exact biological significance of the constants b and k remains unclear. In the present case, k may be regarded simply as the ratio of the percentage change in the number of chaetae to the corresponding change in femur length. A more exact definition depends on knowledge of the time course of determination of chaetae and femur length —exponential, parabolic, or sigmoid—and such is lacking at present. The significance of b is even more ambiguous. As the value of y at $x=1$ in the original system of measurement, it is in these cases a meaningless extrapolation. However, as HERSH (1941) has pointed out, since our units of measurement along the X-axis are arbitrary, we can select any point along this axis as the origin of a new axis parallel to and coinciding with the old one, and if in so doing we select an origin such that $x=1$ has biological significance, then the values of b acquire greater meaning. In the present case we can set $x=1$ at the smallest femur length held in common by all the genotypes studied with respect to a given system. The values of y at $x=1$ as read from the figure may then be regarded simply as indices of the bristle-forming abilities of these genotypes in the neighborhood of the smallest size at which all show extra bristles.

It is clear that in the present case, the effects of a given set of genes on b and k vary according to the region under observation. When the scutellar and wing hairs and the dorsocentral bristles are considered, k remains relatively constant while b varies according to the genotype. The modifiers of the wing and

scutellar hairs studied in connection with *se h* also altered b without significantly affecting the value of k. In the scutellar bristles there is a direct relationship between the magnitudes of b and k; this is probably the first time such a relationship has been described. If we render the bristle data comparable with the hair data, by subtracting four from all means, the dorsocentral data approximate an inverse relationship between the magnitudes of b and k, a type of relationship first described by HERSH (1931), while for the scutellar bristles, k remains constant with increasing magnitudes of b, as in the scutellar and wing hairs. The latter situation is an interesting example of how in a group of related cases fundamental similarities in the manner of increase of two measurements may be obscured by the initial value of one of the measurements. Finally, the sex-comb teeth fail to show any consistent relationship between slope and intercept values. The possible significance of these findings in relation to correlated growth problems will be discussed later.

The foregoing considerations have been directed primarily at the second of the two questions raised in the introduction—namely, the manner of inheritance and interaction of regression coefficients. The first question concerned the types of embryonic reactions revealed within the various chaetae-forming regions, after all the genotypes have been equated to a common size. In the analysis of such a question one is primarily concerned with bringing out such fundamental similarities and differences as may exist between the various systems. The following attempt at analysis will be based on the assumption that the effects of the various factors in combination would be fully predictable from full knowledge of their effects when acting separately. DANFORTH and DE ABERLE (1927) and WRIGHT (1941) have drawn attention to an alternate possibility, "the gene substitutions may bring about not only quantitative differences along a chain of reaction but also transmit differences in specificity. It is possible to dismiss any interaction effect as merely an inexplicable manifestation of specificity, an emergent in the sense of Lloyd Morgan" (WRIGHT 1941, p. 321). While such specificities should be postulated only as a last resort, the possibility of their existence, which in the present imperfect state of our techniques can be neither confirmed nor denied, must be borne in mind. Another conceivable cause of apparent irregularities in interaction, applying particularly to a form like Drosophila with highly mosaic development, is that just as there exist demonstrable differences from region to region in the type of interaction observed, so there are intra-regional differences; the evaluation of interaction effects within, for example, the dorsocentral area is thus the average of several different types of interaction and as such of course does not fall into any simple scheme. These two possibilities will be touched on later.

In consequence of the type of regression observed, it makes no great difference to the picture of interaction effects in the wing hairs, scutellar hairs, or scutellar bristles what particular size is used as a reference point, so long as it is within the range in which all genotypes show extra chaetae. This is not true of the dorsocentral bristles and sex-comb teeth. Table 2 shows the calculated values for wing hairs, scutellar hairs, and scutellar bristles in the various

genotypes at an appropriate femur length. Differences between these values and the corresponding values given in the earlier paper are due to the reduction to a common size, to differences in newly arisen modifying factors, to the fact that these experiments were carried out at 25°C rather than at 24°C as in the preceding work, and to chance.

TABLE 2

*Calculated values for the number of extra wing and scutellar hairs and scutellar bristles at the femur length indicated.*

| CHARACTERISTIC | FEMUR LENGTH ($\mu$) | *pyd* | *se h* | *y Hw* | *y Hw;se h* | *y Hw;pyd* | *se h pyd* | *y Hw;se h pyd* |
|---|---|---|---|---|---|---|---|---|
| Wing hairs | 500 | — | 5.12 ±0.95 | 5.88 ±0.38 | 32.39 ±1.48 | 9.74 ±0.42 | 8.18 ±0.37 | 40.46 ±0.63 |
| Scutellar hairs | 525 | — | 22.79 ±0.53 | 3.93 ±0.23 | 41.21 ±1.25 | 4.78 ±0.26 | 35.55 ±0.39 | 50.29 ♂1.17 |
| Scutellar bristles | 525 | 0.191 ±0.066 | 0.070 ±0.086 | 0.098 ±0.090 | 1.605 ±0.160 | 0.564 0.095 | 0.399 ±0.077 | 2.347 ±0.103 |

The wing hair data, equated to an average femur length of 500$\mu$, may be considered first. Although *pyd* acting separately has no effect on the wing hairs, in combination with either *h* or *Hw* it is a strong modifier of hair number, and in each case to about the same extent. The effects of *pyd* when acting separately are therefore either sub-threshold or else can only be expressed in the presence of *h* or *Hw*. *Hw* and *h* in combination interact very strongly. If some arbitrary value be assigned to *pyd*, it is obvious that the effects of *Hw*, *h*, and *pyd* are more multiplicative when the genes are acting in combinations of two than when all three are together. Probably the simplest hypothesis of factor interaction which brings regularity into these data is an adaptation of the inverse probability transformation which has been described by WRIGHT (1920) and WRIGHT and CHASE (1936). We may assume that at this intermediate fly size the mean number of hairs which can appear on the second longitudinal wing vein has an upper limit in the neighborhood of 45—that is, not much above the number present in *y Hw;se h pyd*. It is an observable fact that when such a number is present, hairs are more or less regularly spaced along the entire length of the vein, and that when hair number rises much above this there is a tendency for hairs to be located on small diverticula off the main vein, or on thickenings of the vein. This fact introduced difficulties into the determination of wing hair number. In the adaptation of WRIGHT'S scheme it is assumed that the addition to the genotype of a given factor tending to increase the number of wing hairs does not have the same phenotypic effect in all combinations of such factors, but has a relatively small effect when acting near the ends of the range, and a greater phenotypic effect near the center, with the exact relation between the "genetic determination" as abscissa and

the phenotypic effect of a vanishingly small increase in "genetic determination" as ordinate approximating in appearance a normal frequency curve. By "genetic determination" is meant the sum total of those genetic effects which tend to evoke hairs on the wing vein. It follows that the relation between "genetic determination" and phenotype, derived from summing the area under the curve to the left of any "genetic determination," is given by the ogive of the normal frequency curve, with the upper asymptote at 45 hairs. "Genetic determination" is measured in terms of $\sigma$ units, with practically the entire range of effective dosage being included in a spread of $6\sigma$. To establish the "genetic determination" corresponding to a particular phenotype, one first calculates the fraction of the maximum possible phenotype which this particular phenotype represents, and then, using a table of normal curve

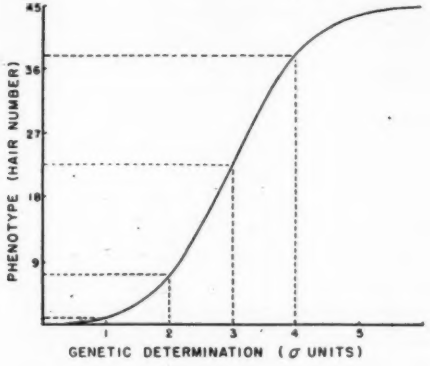


FIGURE 2.—The assumed relation between "genetic determination" (as defined in text) and the number of hairs on the second longitudinal wing vein. It is apparent that a given increase in "genetic determination" (for example, one $\sigma$ unit) has a much larger phenotypic effect in the middle of the scale than toward either end.

areas, determines at what abscissal value in terms of $\sigma$ units from the left end of the normal frequency curve such a fraction of the total area is reached. Conversely, the phenotype resulting from a given "genetic determination" is measured in terms of the area included under the curve between $0\sigma$ and that particular $\sigma$ value from the left end. Genetic effects are assumed to be additive along the abscissa. This hypothesis is illustrated in figure 2.

Since *pyd* has no visible effect when acting separately, it is necessary to assign an arbitrary $\sigma$ value to it. Making this value equal to $.3\sigma$ units, and deriving from the data a value of $1.88\sigma$ for *Hw* and $1.79\sigma$ for *h*, we get the following results for the four combinations:

| | *y Hw;se h* | *y Hw; pyd* | *se h pyd* | *y Hw; se h pyd* |
|---|---|---|---|---|
| Observed $\sigma$ value | 3.58 | 2.21 | 2.09 | 4.27 |
| Expected $\sigma$ value | 3.67 | 2.18 | 2.09 | 3.97 |
| Expected phenotype | 33.71 | 9.27 | 8.18 | 37.53 |

Comparison of the expected phenotypes with those given in table 2 reveals only one significant difference, in *y Hw; se h pyd*. Even closer agreement could be obtained by allowing for the probable departures of the primary phenotypes from the " true" phenotypic values, as the result of chance and modifying factors. Moreover, there is no *a priori* reason for assuming the relation between the strength of the "genetic determination" and the effect of a small increase in this determination to be exactly symmetrical; a slight positive skewness could help account for the *y Hw;se h pyd* discrepancy.

The phenotypic interactions seen in connection with the scutellar hairs are somewhat similar to those met in the wing hairs. Thus, *pyd* has no effect in the absence of *Hw* or *h*, but combined with either acts as a positive modifier. Moreover, the relative effect of *pyd* is greatest in combination with *h*—that is, at an intermediate phenotype. However, if some reasonable value be assigned to the maximum mean number of scutellar hairs, and an attempt be made to treat the data in the fashion employed for the wing hairs, significant departures from expectation are observed. Polychaetoid interacts more strongly with *se h* than with *y Hw*. Now, it is well known that mutations affecting chaetae number characteristically have a region or regions of maximum effect, with the departure from wild type decreasing as distance from that center increases (*cf.* NEEL 1940b for discussion and references). The scutellar hairs in *y Hw* flies tend to be on the anterior part of this region, while in *se h* they are usually more posteriorly situated (NEEL, 1941). We may therefore surmise, following the discussion of p. 59, that the "invisible field" of *pyd*, whose existence we deduce from the interaction of *pyd* with the other factors, more nearly coincides with that of one of these factors than the other, and so differences in the phenotypic interactions result. The general type of interaction would still be as in the former case.

The scutellar bristles, equated to a femur length of 525$\mu$, give a quite different picture of factor interaction than do the wing or scutellar hairs. Extra bristles are present in all three primary genotypes, with the seriation *pyd*>*y Hw*>*se h*. However, the combination of *Hw* and *h* exhibits a very significantly greater departure from wild type than either of the other two combinations of two factors, and this marked interaction is maintained in the combination of all three factors. WRIGHT (1935) has described on theoretical grounds a type of factor interaction where the phenotypic departure from an optimum (in this case, wild type) varies as the square of an additive primary genetic effect. The effects of *pyd* in combination with *Hw*, *h* and *Hw;h* show a fair approximation to such a scheme. If we accept the values observed in *pyd*, *se h*, *y Hw*, and *y Hw;se h* as a good approximation to the "true" values, then the comparison between observed and expected results is as follows:

| Genotype: | *pyd* | *se h* | *y Hw* | *y Hw; se h* | *se h pyd* | *y Hw; pyd* | *y Hw; se h pyd* |
|---|---|---|---|---|---|---|---|
| $\sqrt{\chi_{observed}}$: | .44 | .26 | .31 | 1.27 | .63 | .75 | 1.53 |
| $\sqrt{\chi_{expected}}$: | | | | | .70 | .75 | 1.71 |
| $\chi_{expected}$: | | | | | .490 | .563 | 2.924 |

There is a good correspondence between observation and expectation in two of the three cases. In view of the roles of chance and modifiers in determining

bristle numbers, it seems entirely possible that, for example, the observed values for *pyd* and *y Hw;se h* are too high by an amount equal to one times the standard error of their determinations. Assuming, then, a value for *pyd* of 0.126 and for *y Hw;se h* of 1.462, the comparison between "observed" and expected becomes as follows:

| Genotype: | *pyd* | *se h* | *y Hw* | *y hw; se h* | *se h pyd* | *y Hw; pyd* | *y Hw; se h pyd* |
|---|---|---|---|---|---|---|---|
| $\sqrt{x_{observed}}$: | .35 | .26 | .31 | 1.21 | .63 | .75 | 1.53 |
| $\sqrt{x_{expected}}$: | | | | | .61 | .66 | 1.56 |
| $x_{expected}$: | | | | | .372 | .436 | 2.434 |

This is a somewhat better fit. No other simple scheme has been found which results in so good a comparison between observation and expectation. We may therefore tentatively regard the scutellar bristle results as produced by a multiplicative interaction between two factors, with a third factor acting additively, the phenotype increasing as the square of these additive primary effects. CHARLES (1938) has reported a comparable interaction of genes affecting the spotting pattern in the mouse: on a suitably transformed scale three of the four factors investigated appeared to act additively, while the fourth had a multiplicative effect.

In both *y Hw* and *se h* the frequency of extra scutellars was so low at intermediate and small femur lengths that the regressions could be derived over only a relatively limited range in fly size. This picture of factor interaction is therefore to be derived from the data only at relatively large fly sizes.

In view of the proximity of scutellar hairs and bristles, both were equated to the same size in the above treatment, a size in this case dictated by the scutellar bristle data, in order to facilitate the establishment of any possible interrelationships. None was found.

In the case of the dorsocentral bristles and teeth in the sex-comb, the exact picture of gene effects which one obtains depends to a much larger extent than in the preceding on the size at which one evaluates these effects. This is obvious from figure 1. However, the general features of the interactions observed in the dorsocentrals are the same at all sizes, in spite of important differences in detail. Although extra dorsocentrals were present in all three primary genotypes, in *se h* they were so sporadic that a regression could not be derived. The supernumerary dorsocentrals evoked by *pyd*, and, more rarely, by *se h*, are quite restricted in distribution, usually falling in line with the normal dorsocentrals, while those associated with *y Hw* have a more general distribution. In spite of the wide differences in the *pyd* and *se h* phenotype, the interaction of either with *y Hw* results in a very similar, phenotypically super-additive end effect. On the other hand, the interaction of *se h* with *pyd* results in a fly with fewer dorsocentrals than *pyd*. The fact that a similar, although non-significant, interaction between *se h* and *pyd* was noted in the earlier investigation makes it probable that this peculiar effect is not due to modifiers in the *se h pyd*, *pyd*, or *se h* strains. While this suggests competition effects within the relatively limited area in which these factors induce departures from wild type, the application of such a concept is difficult in view of the

strongly positive interaction seen in *y Hw;se h pyd*. No consistent scheme relating all these effects has yet been developed.

Similar but even more complicated relations are apparent in the sex-comb teeth. Thus, *y Hw* and *se h* increase slightly the number of teeth in the sex-comb, but the combination of these two has significantly fewer teeth than normal. Polychaetoid flies also have fewer teeth than normal. However, the combination of *y Hw;se h* with *pyd* results in a fly with significantly more teeth than wild type.

It is obvious from the foregoing that, as nearly as can be judged from the phenotypic picture, the dynamic relations of these three genes differ significantly according to the part of the body under observation, even though the characteristic studied is more or less comparable in all regions. The question of "specific interactions," mentioned above, emerges most clearly in connection with such apparently irregular results as those pertaining to the sex-comb teeth. A related question of great importance to the ultimate interpretation of the apparent regional differences in factor interaction is this: do the factors involved have the same primary effects in all regions of the body, with the different end effects imposed by local circumstances, or does one factor have several different and independent roles to play? In the latter case there is of course no reason to expect the same type of interaction in all systems in which the factor plays a role, nor to attempt to interpret the phenotypic interactions seen here so that at one stage they fit into a common pattern (for a fuller discussion of this question see SILOW 1939 and NEEL in press). Evidence as to the physiological versatility of a factor may be derived in various ways, and particularly from a study of multiple allelic series of factors with pleiotropic effects. Unfortunately for the present problem, mutations resulting in *Hw* and *pyd* have been reported but once. However, the $+^h$ locus mutates relatively frequently. Most of the alleles which have been reported are similar to the one studied here, but one ($h^{42a19}$) is characterized by a restriction of supernumerary chaetae to the wing (NEEL 1942b) and another ($h^2$) by a similar restriction of extra hairs to the wing and in addition by cylindrical bristles with javelin heads (Drosophila Information Service 9). (The location of the "javelin" mutation at 3–19.0 raises a question as to whether $h^2$ may be the result of a small chromosomal aberration involving the javelin locus.) The fact that the $+^h$ gene may mutate in what appear to be qualitatively different directions may be taken as evidence of some biochemical independence of the various effects of the factor, although obviously decisive evidence is lacking. For the present the question of the relationship between the effects of these factors must be left open. WRIGHT (1927, 1941) and RUSSELL (1939) have described a somewhat similar case in the guinea pig, where the interaction of factors affecting pigmentation appears to be different in the epidermis, eye, and hair, and have interpreted it in terms of regional threshold differences. This explanation has been challenged by SILOW (1939). It is difficult to see how a similar explanation could be applied here.

THE RAPID ACCUMULATION OF MODIFIERS OF FLY SIZE IN THE STRAINS

It was suggested above that in these strains minor modifiers of bristle number had arisen with a relatively high frequency. The same appears to be true of modifiers of fly size. The relation between the concentration of the yeast suspension on which the flies were grown and mean femur length, when 100 eggs were introduced into a vial, is shown in figure 3. As yeast concentration increased above 10 percent there was no significant increase in femur length,

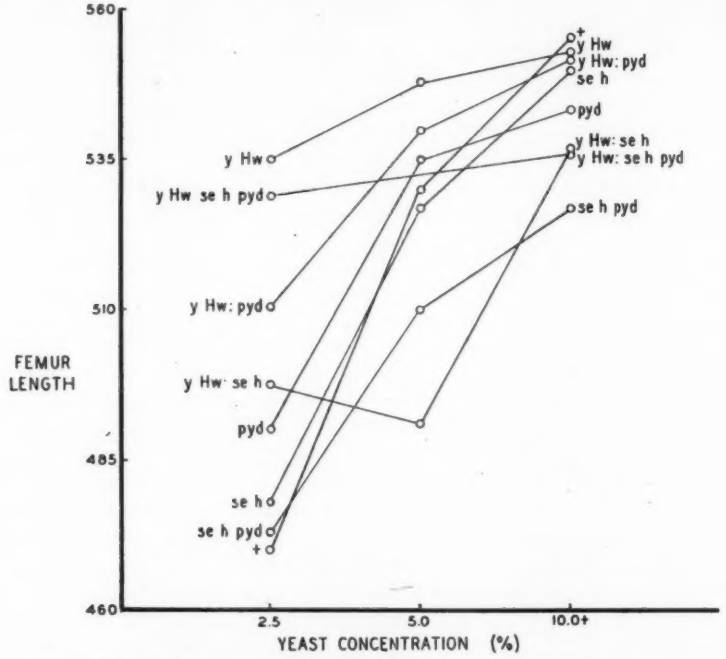


FIGURE 3.—The relation between food level and fly size in eight different strains of Drosophila. Details in text.

and for this reason the data on concentrations down to and including 10 percent have been averaged together. The "10+ percent" size seriation so obtained may be compared with the seriation observed at a yeast concentration of 15 percent in the earlier work. The differences between the two series are so great that either the determinations were subject to large errors or else genetic differences had appeared in the strains in the eight to ten months (14–18 generations) elapsing since the earlier work. Culture conditions were so carefully standardized that the first explanation appears insufficient. We have then to fall back on the second explanation and recognize a rapid appear-

ance of modifiers of size. These modifiers may be either positive (for example, the *y Hw;se h* stock) or negative (the wild type strain); there is no evidence as to whether or not they are the same ones concerned with chaetae number. Some four months after the constants had been determined in the wild type strain, it was found to contain a "mutability stimulating" factor (NEEL 1942a). The majority of minor mutations produced over and above the normal rate under the influence of this factor were undoubtedly deleterious. There is no evidence as to how long the factor had been present in the strain prior to its discovery. If it had already been present in the strain for some time previous to the determination of the constants, this could account for the very considerable decrease in the size of the $+^{Or}$ flies. However, the *y Hw* strain was also found to carry the factor, it having been introduced in crosses of this strain to the Oregon line, and yet the *y Hw* flies showed no size decrease. There was no evidence for the presence of the factor in other strains.

The significance of the size differences between strains was discussed in the earlier paper.

### THE RELATION BETWEEN NUTRITION AND SIZE IN THE VARIOUS GENOTYPES

It is obvious from figure 3 that the seriation of genotypes with respect to mean femur length at 10+ percent yeast concentration is quite different from that at 2.5 percent. Under poor food conditions the four strains with the longest mean femur lengths all contain *y Hw*, while under more favorable conditions the observed seriation is significantly different. A similar tendency was noted when 200 eggs were introduced into a vial, although the data are not so extensive.

All *y Hw* males were the offspring of *y Hw*/++ females; since, due to the poor viability and fertility of the homozygous *y Hw* females, it was necessary to propagate the various strains containing *y Hw* by heterozygous females. In any strain containing *y Hw* there was thus a segregation in each generation for *y Hw* and non-*y Hw* males. In such cases at low food levels the larger size of the *y Hw* males was readily discernible under the microscope. The size disparity was particularly marked in the offspring of *y Hw*/++; *se h pyd* females, the *y Hw;se h pyd* males having an easily detected size advantage over the ++; *se h pyd* segregants. These intra-strain differences are conclusive evidence that the larger size of the *y Hw* males at low food levels is not due to the chance accumulation of similar modifiers in the *y Hw* strains but is a function of *y*, *Hw*, or some closely linked factor.

Among the thousands of mutant types that have been studied in various species of plants and animals in the past 40 years, only a very few have been reported as superior to wild type with respect to viability, fertility, or any other characteristic which could be of adaptive significance. These "favorable mutations" have recently been reviewed by GUSTAFSSON (1941) and will not be further discussed here. However, it should be emphasized that in most of the previously discussed cases, like the present, the mutant type is superior to the wild type only under certain special conditions, which are yet not so spe-

cial but that they could conceivably arise in nature. Such differential responses of mutants are of particular interest from the standpoint of the theory of "preadaptation."

BRIDGES (1939) has very briefly mentioned the greater-than-normal viability of a yellow allele ($y^{34e}$). NILSSON (1931) described a case of increased resistance to high temperature in a *y w* strain. BURKART and STERN (1933) drew attention to the high viability of the Blond translocation, characterized by a reciprocal interchange between the tips of the first and second chromosomes. This may be related to an involvement of the *y* locus. If the present case is due to the yellow allele, this would constitute another possible instance of mutation at the $+^{y}$ locus resulting in a phenotype which under certain conditions had a selective advantage over wild type. The suggested relatively high frequency of "favorable mutations" at this locus deserves further study. On the other hand, it would be equally significant if the factor responsible was *Hw*, since DEMEREC and HOOVER (1939) have shown that this is a short duplication. NILSSON (1932) has reported that the Bar mutant, also due to a small duplication, is characterized by high viability under poor food conditions. These may therefore be cases where under certain conditions new chromatin has a selective advantage; such events provide a mechanism for intrachromosomal evolution. Unfortunately, in the present case it does not appear possible to reach a decision between these two alternatives, since the *Hw* duplication first arose in combination with this particular *y* and is so closely linked that it has not been observed to separate through crossing over.

## DISCUSSION

NEEDHAM and LERNER (1940) have pointed out the need for distinguishing between the actual relative growth of parts in relation to the whole or other parts (heterauxesis) and relative growth relationships between groups or individuals compared at maturity or any other given stage of development (allometry). A complication in many of the existing attempts to compare growth relationships in related organisms, or to study the inheritance of growth constants, has been the failure to distinguish between these two phases of relative growth. A further complication has been an almost complete lack of knowledge of, or control over, the gene differences contributing to the growth differences obtaining in the forms under analysis (e.g., LERNER 1937, 1941; GREEN 1933). It is not immediately clear whether the present data are entirely comparable to the usual phenomena included in studies of correlated growth. Certainly there is a superficial difference between increase in the numbers of such specialized structures as chaetae and the growth of an organ. However, earlier studies (NEEL 1940b) have shown that in *pyd* flies there is a close correlation between the number of dorsocentral bristles present and their total length—that is, each bristle number is distinguished by a more or less characteristic range in total bristle lengths. If this relationship is general for the various groups of chaetae studied, then correlating femur length with

chaetae number amounts to correlating size with the amount of chitin, etc., incorporated into certain special structures in various regions of the body. Among the various growth studies the present data are thus most nearly comparable to the type of investigation reviewed by NEEDHAM (1934), concerning the relation of chemical changes to increase in body size, with the difference that the substance under investigation here is distributed in the form of discrete units. Considered in this light, the data are quite pertinent to the problem of relative growth, since they constitute a unique study of the effects of single gene substitutions on the constants of allometry. The salient feature of the findings is the fact that the picture obtained of the genetic control of growth constants depends largely upon the particular structure studied. However, in the appearance of *extra* structures, on the basis of limited data, one relationship does appear to stand out: in some systems gene substitutions alter the magnitude of the departure from wild type without changing the ratio of the percentage increase of character and body size.

The differences in the various body regions in the relation between the magnitudes of slope and intercept in the different strains appear to be related to the nature of the phenotypic interactions. In those three cases agreeing in the relation between size and the number of extra chaetae, the picture of interaction effects is somewhat similar: primary genetic effects are often additive, although the exact relation between phenotype and this additive effect probably differs in the three cases. Such additive effects imply a similarity of gene action, although we are still in the dark as to what this similarity entails. Conversely, the non-additive interactions noted imply a regulation of the production of a given characteristic by different means. Such non-additive interactions are particularly prominent where the dorsocentral bristles and sex-comb teeth are concerned, and these same structures also showed an irregular relation between slope and intercept in the regressions. The question of a "pleiotropic" as compared with a "single" action of the factors studied has already been discussed.

A great deal of work has been performed on the inheritance of quantitative characters, and criteria have been developed for attempting to distinguish between an arithmetic and geometric action of the factors involved (*cf.* CHARLES and SMITH 1939). Most of these studies have utilized plant material and have dealt with the interactions of large blocks of chromatin rather than single or a relatively few factors. The length of the life cycle and the paucity of genetic markers in most plants render a more precise approach difficult. Nevertheless, if the present data are indicative of the types of phenotypic interactions involved in the production of quantitative characters, then it seems to the author that in more complex cases relatively little light is shed on the nature of such inheritance by determining whether an unknown number of factors adhere more closely to a geometric than to an additive type of interaction. Rather, the admittedly much more difficult analysis of the effects of single gene substitutions is in the end the approach which must be used. CASTLE (1941) has recently summarized the relatively few attempts to do this

for mammalian material. The results are fully as complicated as those reported here for Drosophila.

HARLAND and ATTECK (1933) and MULLER and PONTECORVO (1942) have attempted to explain the appearance of aberrant characteristics in crosses of similarly appearing organisms with the suggestion that in related forms similar phenotypes may be the result of somewhat different genetic systems. The present data furnish several examples of the control of a similar phenotype by different factors, and departures from either parental form in derived types. Thus, at large sizes *y Hw* and *se h* are similar as regards the number of hairs on the second longitudinal wing vein and the number of teeth in the sex-comb. Yet the compound *y Hw; se h* has approximately six times as many wing hairs as either *y Hw* or *se h*, but significantly fewer sex-comb teeth. At this rate, fairly large phenotypic aberrations could be obtained in a cross where the parental forms differed by a relatively few factors.

While it appears probable that "concealed genetic variability" of the type recently stressed by DOBZHANSKY et al. (1942) is the major cause of the phenomena of heterosis, a possible secondary role of factor interactions such as have been described above cannot be disregarded entirely.

## SUMMARY

A study was made of the relation between body size and the number of five different kinds of chaetae present in *Drosophila melanogaster* males of the following genotypes: wild type, *pyd*, *se h*, *y Hw*, *y Hw; pyd*, *y Hw; se h*, *se h pyd*, and *y Hw; se h pyd*. In each case the regression of number of chaetae on femur length is adequately represented on an arithmetic plot by a straight line. The five families of straight lines, each representing the relation of a particular kind of chaetae to size in the eight strains, show different relations of slope and intercept. In each of three families the various strains differ in respect to the magnitude of the intercepts of their regression lines, but the ratio of percentage change in number of extra chaetae to percentage change in femur length remains constant within the limits of chance variation. In the other two families of lines much more irregular effects were noted.

The apparent nature of the phenotypic interactions of the factors depends to a considerable extent on the fly size at which these effects are evaluated. In the same three cases mentioned above, the data can be interpreted as indicating that primary genetic effects are mainly additive, but that the subsequent relation of effect to phenotype is different in each case. In the other two cases no satisfactory scheme of factor interaction has yet emerged.

Under poor food conditions male flies containing *y Hw* showed a significantly smaller reduction in mean fly size than did flies not containing this combination of factors.

The relation of these findings to the problem of correlated growth is discussed.

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# ABSTRACTS OF PAPERS PRESENTED AT THE 1942 MEETINGS OF THE GENETICS SOCIETY OF AMERICA

NEW YORK CITY, DECEMBER 29–31, 1942

B. P. KAUFMANN, *Secretary*
*Carnegie Institution of Washington*
*Cold Spring Harbor, New York*

ATWOOD, S. S., and J. T. SULLIVAN, U. S. Regional Pasture Research Laboratory, State College, Pennsylvania: *Inheritance of a cyanogenetic glucoside and its hydrolyzing enzyme in white clover.*——Inbred lines were found which gave uniform o or + reactions to the picric acid test for cyanogenetic glucoside. An $F_1$ between o and + lines was + throughout, and the $F_2$ segregated 351 (+) : 256 (o). By the chi-square test this ratio fits 9:7, suggesting two independent factors conditioning the presence of glucoside. The backcross (B), however, segregated 204 (+) : 295 (o), a ratio which doesn't fit 1:3, expected on the same hypothesis. A test of separate families showed that some $F_2$ fitted 3:1 better than 9:7, and all B fitted 1:1 or 3:5 better than 1:3. This would result if the original o parent was homozygous recessive for one factor and heterozygous for the other. Separation of the $F_2$ and B families on this basis gave a consistent fit throughout. An $F_3$ obtained by backcrossing 21 $F_2$ plants (all +) with their original o parent showed ten families segregating 3:5, six segregating 1:1, four segregating 3:1, and one with only + plants; the expected ratio was 8.9:5.1:4.4:2.6. Subsequently in another cross chemical preparations of the glucoside and its enzyme were added separately to distinguish plants having only enzyme or only glucoside. A good fit to dihybrid ratios was obtained in most $F_2$ and B families, indicating that the independent factors previously postulated were specific for the glucoside and its enzyme. Deviations from expected in some families suggested modifying genes. Part of the population also was segregating 3:1 for a factor conditioning white leaf markings; this gene was independent of those for glucoside and enzyme.

AVERY, A. G., and A. F. BLAKESLEE, Dept. of Botany, Smith College, Northampton, Mass.: *Mutation rate in Datura seed which had been buried 39 years.*——It was earlier shown that the mutation rate in Datura is greatly increased by the aging of seed when stored under ordinary laboratory conditions, the increase being about 1 percent for each year of aging up to nine or ten years, beyond which time the seed failed to germinate. A comparable increase in rate of mutation was not found in plants grown from seed which had been buried in the ground. In 1902, seeds of a large variety of plants were buried near Washington as part of a germination experiment by the Department of Agriculture. In 1941, 367 seeds of this 39 year old Datura material were sent us. They produced 358 seedlings or 97.5 percent germination. Their pollen showed that very few mutations responsible for pollen abortion had taken place. In the summer of 1942, $F_2$ progenies were grown from 78 of these plants. Two of them showed segregation for pale-leaved types and one for a recumbent type

called "lazy." One progeny segregated for a type which resembled the $2n+15\cdot16$ primary. This was found to have only the normal number of chromosomes and may thus be tentatively presumed to be due to a recessive mutation which with the other three gives a mutation rate of 5.1 percent. This is distinctly higher than that obtained from controls in our standard line but is much lower than from seed "aged" under laboratory conditions.

BEERS, C. V., and W. G. SHULL, University of Southern California, Los Angeles, Calif.: *Hemophilia, heredity and treatment.*——Treatment is successful in preventing the expression of genetic hemophilia. DR. EMERALD J. STEEN of Fullerton, California, states that the hemophilic medicine prepared from the egg white and potassium bromide formula of DRS. TIMPERLEY and CLARK of SHEFFIELD UNIVERSITY, England, has been most successful in combatting this disease in the case of the propositus. The clotting time has been brought from 60–90 minutes down to 10–15 min. In this family the manifestation of the disease suggests the ratio expected for a mendelian sex linked character. There is no history of hemophilia in this family previous to the third generation, and the facts point to the probablity that the gene for hemophilia arose as a mutation in a woman of the second generation. The propositus, his brother, and two uncles are hemophilics.

BERGER, C. A., and E. R. WITKUS, Fordham University, New York, N. Y.: *A possible source of new evidence on the difference between mitosis and meiosis.* ——In the periblem of root tips of spinach, in addition to cells having the normal diploid number of 12 chromosomes, larger cells are regularly found which are tetraploid or octaploid. This polyploid condition (polysomaty) arises by each chromosome reproducing itself twice during resting stage. The prophase of the first mitosis after such a double reproduction shows sister chromosomes closely paired and relationally coiled. Each of the paired chromosomes consists of two chromatids and thus the pair is similar to a bivalent chromosome of meiosis. At later prophase as the paired chromosomes contract, a stage is reached similar in appearance to meiotic diakinesis and called "somatic diakinesis" by some authors. No chiasmata are formed, and the paired chromosomes at metaphase are completely separated from each other while retaining a definitely paired association. This paired chromosome condition is manifested only in the first mitosis after double chromosome reproduction. Polyploid cells which have undergone a complete mitotic cycle subsequent to attaining a certain degree of polyploidy do not show the paired chromosome condition at future mitoses unless another period of double reproduction occurs. A detailed comparative study of polysomatic prophases showing pairing and of meiotic prophase is being made in the hope of obtaining decisive evidence on some of the assumptions inherent in current theories of the difference between mitosis and meiosis.

BERGNER, A. D., and A. F. BLAKESLEE, Carnegie Institution of Washington, Cold Spring Harbor, N. Y., and Smith College, Northampton, Mass.: *Geographical distribution of chromosomal types in Datura metel.*——This species is

widely distributed throughout the warmer regions of the world. Type 1 is the only widespread chromosomal type including 50 of the 61 races studied: all those from the western hemisphere, the Hawaiian Islands, Turkey, and Africa. It is the predominant type east of 60° east longitude. It was not found in China or Japan. It is not linked with flower color (purple, lavender, yellow, white) nor with type of corolla (single, double). Of the four other, apparently endemic, chromosomal types, one came from northern India, one from Java, another from China, and the fourth from both Java and China. Types 2 and 3 are combined in cultivated races from China. All differ from type 1 by a single interchange between two non-homologous chromosomes. *Inter se* crosses show that only five chromosomes are involved in these interchanges, one chromosome being involved in three and another in two interchanges. In *D. stramonium* also not all chromosomes are involved in interchanges with equal frequency. Type 1 is probably the oldest from which the others were derived. Types 2, 3 and 5 appear to have arisen in white, single races; type 4 in a purple double flowered race. Distribution of races according to flower color is distinct in southeastern Asia and is independent of chromosomal type. It is also suggested in Africa. The absence of type 1 from China suggests that *D. metel* had spread to China after types 2 and 3 had originated.

BOZEMAN, M. L., University of Pennsylvania, Philadelphia, Pa.: *Effects of irradiation on oöcytes of Sciara.*——The oöcytes of *Sciara ocellaris* develop almost synchronously. The first meiotic metaphase occurs at about 24 hours after eclosion of the adult female. Anaphase movement begins at about 27 hours, is arrested at about 30 hours and begins again after fertilization. The eggs are laid at 55 to 60 hours. Adult females were irradiated at ages from 20 to 55 hours after eclosion, and smears of the $F_1$ salivary gland were examined for induced aberrations. Sensitivity to irradiation is first detectable in larvae developing from eggs treated when the chromosomes begin to shorten and move to the metaphase plate. Sensitivity increases through metaphase and early anaphase, then decreases during the arrested stage. Just before fertilization sensitivity again increases.——Distribution of "breaks" per cell is random, but recovered rearrangements are not randomly distributed among chromosomes. Aberrations found are: inversions, deletions, transpositions and repeats. Only one translocation was found among 116 aberrations. This deficiency of translocations might be expected, since the chromosomes are well separated during the first meiotic division. The occurrence of repeats is of special interest from the standpoint of the mechanism of rearrangement. The ratio of repeats to other aberrations is highest in prophase, lower in metaphase, lowest in early anaphase and slightly higher in later anaphase. The variation in sensitivity, the high frequency of intrachromosomal rearrangements and the unequal distribution of types of aberrations when different stages of meiosis are treated indicate that rearrangement is not delayed until fertilization, but occurs shortly after irradiation.

CARSON, HAMPTON L., University of Pennsylvania, Philadelphia, Pa.: *Cytological analysis of natural populations of Sciara impatiens.*——Examination of

salivary gland chromosomes of the offspring from 157 wild females collected in the eastern United States reveals the following variations: (a) 12 inversions, (b) 13 small changes of the duplication-deficiency variety; apparently both types are distributed at random among the four chromosomes. Three inversions are cytologically terminal and two hypothetical intermediate gene arrangements apparently involve the same condition. Six of the small changes appear to be terminal deficiencies, which may be homozygous, and several deficiencies have presumably arisen in conjunction with inversions. Most of the aberrations are widely distributed in the population, and none produces detectable phenotypic effects. Conditions in this species differ from those in *S. ocellaris* and *S. coprophila* in that numerous gross chromosome rearrangements are found, but agree with those species as regards frequency of small changes. With respect to the frequency of gross rearrangements, *S. impatiens* resembles Drosophila, but (like other species of Sciara) differs from the latter genus in the manifestation of numerous minute modifications.

CREIGHTON, HARRIET B., Wellesley College, Wellesley, Mass.: *Color pattern and doubleness in Petunia flowers.*——The proportions of colored and white tissue in flowers of certain diploid petunias changes during the outdoor growing season. Plants that have flowers in midsummer with small amounts of white have flowers in early fall with half or more of the corolla white. In conjunction with inheritance studies of this type of flower pattern a few studies have been made of the effects of mineral nutrient conditions and of different photoperiods on the expression of the character. Rooted cuttings from one plant were grown in well fertilized soil and in sand cultures where partial and complete deficiencies of nitrogen, phosphorus, potassium, sulphur, magnesium and calcium were obtained and a partial deficiency for iron, zinc, manganese and boron. At the end of six months the flower color patterns of plants in soil and in any of the sand cultures were indistinguishable, although the vigor of the plants had been markedly affected by the mineral nutrient supply. For an additional three months all plants in sand were given a nutrient solution with high nitrogen and potassium. The vigor of the plants increased and with this the proportion of colored corolla tissue until almost all of the flowers were completely colored.——Photoperiods of eight hours and 16 hours followed by 16 hours and eight hours of darkness, respectively, had no effect on flower color pattern.—— Doubleness of flowers in certain tetraploid petunias seems to be controlled by two dominant genes. No plants homozygous for either gene have been found.

DOVE, W. FRANKLIN, ELIZABETH F. MURPHY, and ROBERT V. AKELEY, Biological Laboratory, Me. Agr. Exp. Sta., University of Maine, Orono, Me., and Bureau of Plant Industry, U.S.D.A., Beltsville, Md.: *Varietal differences and inheritance of vitamins C and A in potatoes.*——By means of a rapid titrimetric technique for the determination of ascorbic acid, 22 varieties of important American commercial table varieties, 15 promising seedlings, 14 German and two wild varieties of potatoes were found to contain, soon after digging, $30 \pm .67$, $29 \pm .88$, $30 \pm .89$, $22 \pm 2.38$ mgms of ascorbic acid per 100 grams. Duplicate tests differed by 1.45 mgms; replicate plots by 3.1 mgms. Thirteen

varieties grown in two different localities gave an r of $+.79 \pm .07$. Variety values ranged from $20 \pm .70$ for a seedling to $40 \pm .40$ for Voran.——There appeared a multimodal distribution of ascorbic acid values. High and low varieties resulted from the same cross (Katahdin $35 \pm .37$ and Chippewa $23 \pm .20$). The highest-testing seedling ($40 \pm .35$) came from two high parents, Katahdin and Rural New Yorker ($33 \pm .96$). High-testing parents tended to produce medium to high offspring. Medium-testing parents tended to produce medium to low offspring. One low-testing parent produced a low-testing $F_1$ seedling. Ascorbic acid values were not associated with tuber volume, shape, eye depth, starch content, or earliness. All pink-eyed and pink-skinned varieties, however, were below 30 mgms in ascorbic acid values,—that is, 12 pink-eyed varieties (ten of which were pink-skinned) contained $26 \pm .69$ mgms, while 43 non-pink contained $30 \pm .53$ mgms.——Two varieties of known high vitamin A activity were among the lowest in vitamin C. The Golden variety and a seedling, both with relatively high carotene content, contained $22 \pm .41$ and $20 \pm .70$ mgms of ascorbic acid respectively. Yellow flesh, sometimes indicative of carotene, was associated with both low- and high-ascorbic acid values.

EIGSTI, O. J., and LEONA SCHNELL, University of Oklahoma, Norman, Okla.: *A comparison of colchicine treatment with a glycerine base and a water base.*——Two groups of seedlings of *Vinca rosea* ($2n = 16$), 1000 in each group, were subjected to treatment with a 1 percent colchicine-in-glycerine and 1 percent colchicine-in-water, respectively. Two-thirds of each group were given five applications, and one-third were given four applications by the brush method. Approximately 5 percent of the plants treated with colchicine-in-glycerine became tetraploid, whereas less than 1 percent tetraploidy resulted in those treated with colchicine-in-water. A higher percentage of chimeras occurred in the colchicine-in-glycerine treatment. There was a slightly greater mortality of the plants subjected to the colchicine-in-water treatment. A 1 per cent solution of colchicine-in-glycerine proved more effective for the induction of polyploidy in Vinca. From a sampling of several hundred plants during the past four years, no naturally occurring tetraploids have been detected.

EIGSTI, O. J., and BARBARA TENNEY, University of Oklahoma, Norman, Okla.: *Range of concentrations and number of applications of colchicine effective for the induction of polyploidy in Vinca rosea.*——Fifteen hundred seedlings of *Vinca rosea* ($n = 8$) were treated with colchicine in a glycerine base to determine which concentration of colchicine (1, 0.75, 0.50, 0.25, 0.1, 0.01 percent) and how many applications (one, two, three, four, five spaced at 12 hour intervals) are most effective for the induction of polyploidy in this species. This method required 30 replicates of 50 seedlings each for treatment, and one series for controls. The seedlings were four weeks old and had two sets of leaves at the time of treatment. Opposite foliar arrangement facilitated the administration of colchicine by the brush method used in this study.——Ten percent of the plants given five applications of 1 percent colchicine developed into tetraploids, the highest percentage of tetraploidy secured from any of the series. No tetraploids were detected in series with four applications or less of .5 percent, and

five applications or less of .25, .1, .01 percent, respectively. The criteria of tetraploidy were stomatal and pollen sizes, verified, in some cases, by chromosomal counts. Periclinal chimeras appeared in all the series except the five series treated with .01 percent colchicine.——Results indicate that five applications of 1 percent colchicine in a glycerine base is the most effective; also that, within the limits of the series used, the concentration of the colchicine preparation and not the number of treatments is the more important factor in inducing polyploidy in seedlings of *Vinca rosea*.

FANKHAUSER, G., and RITA C. WATSON, Princeton University, Princeton, N. J.: *The effectiveness of cold and heat as triploidy-inducing agents in salamanders.*——Refrigeration of salamander eggs beginning immediately after fertilization and lasting for from five to more than 24 hours produces a high proportion of triploid larvae. In amphibian eggs, the second maturation division is arrested in metaphase until fertilization occurs; the treatment presumably prevents it from resuming its normal course. Temperatures from 0° to +4°C seem to be most effective and have produced from 44 percent to 80 percent triploid larvae in three species of salamanders. In *Triturus viridescens*, eggs of certain females actually gave 100 percent triploid larvae, and temperatures as high as +5.5° and +6.4°C still produced 35 percent and 23 percent triploids, respectively.——Last spring 113 freshly fertilized eggs of *Triturus viridescens* were exposed to temperatures ranging from 34.2° to 37.2°C, for from five to more than 50 minutes, and raised at room temperature. Sixty-two (54.9 percent) of the treated eggs developed into larvae. Of these 49 (79 percent) were found to be triploid, the rest diploid. The shortest heat treatments which produced triploid larvae were five minutes at 37° and eight minutes at 36°C. The lowest effective temperature was 35°C.——The rate of mortality among the treated eggs and the percentage of triploid larvae obtained in these heat experiments compare favorably with the results of refrigeration experiments. Both heat and cold are much more effective polyploidy-inducing agents in salamanders than in plants, probably because it is possible to treat all the eggs in exactly the same stage of meiosis and during a period when the chromosome movements are completely suspended.

FANO, U., Carnegie Institution of Washington, Cold Spring Harbor, N. Y.: *Neutron-induced lethals in Drosophila.*——An experiment was carried out to provide additional information on the production by neutrons of genetic changes in *Drosophila melanogaster* sperms. Wild type males were treated with approximately 600 and 1200 neutron units from the COLUMBIA UNIVERSITY cyclotron, and their offspring were tested for dominant and recessive lethals. One neutron unit is energetically equivalent to about 2.5 r X-rays. The frequency of dominant lethals was found to be approximately 73 percent and 96 percent for the two treatments; the same effect is produced by about 3000 and 6000 r X-rays, respectively. Thus the ratio between the energies delivered to tissue by X-ray and neutron doses having equal dominant lethal effect approaches 2; the value 1.5 was reported previously for this ratio by DEMPSTER. Among the offspring of 170 males treated with 1200 units, 998 *ClB* tests were

performed, yielding 60 recessive lethals; this frequency agrees with that reported by ZIMMER and TIMOFEEFF-RESSOVSKI. No "grouping" of lethals was observed in the offspring of individual males, failing to confirm the report of NAGAI and LOCHER. All but one of the lethal-bearing chromosomes were further tested genetically and cytologically. Only two chromosomes were clearly shown to carry two separate lethals, a coincidence that may be attributed to chance; this finding does not agree with the report by NISHINA and MORIWAKI of significant "grouping" of lethals in individual chromosomes. Genetic analysis indicates that more than 30 percent of the recessive lethals are associated with gross chromosomal rearrangements. According to partially completed cytological analysis, this estimate may be slightly increased; no association of lethals with minute deficiencies has thus far been revealed.

GOODALE, H. D., Mount Hope Farm, Williamstown, Mass.: *New extreme variants in a selection experiment.*——Continued selection confined to a stock originating in one mouse with a few white hairs on its forehead and four self-colored females brought a slow increase in head white accompanied in later generations by white on the extremities and belly. After some 22 generations, mice appeared resembling piebalds but breeding differently, rarely having "piebald" children. Judging by past experience, a slow increase in the proportion of "piebalds" may be expected in future generations accompanied by new extremes.——Extreme variants are rare, and only a small proportion reproduce themselves or produce new extremes. Selection, if it is to be effective in changing characters, must be on a scale in each generation sufficient to discover the few extremes which beget new extremes.

GOODRICH, H. B., R. W. HARRISON, N. D. JOSEPHSON, and J. P. TRINKAUS, Wesleyan University, Middletown, Conn.: *Three genes in Lebistes reticulatus.* ——These genes of the "guppy," *Lebistes reticulatus*, are all autosomal recessives to the wild type. Two, Blond and Golden, produce fish which are lighter in color than the wild type. In Blond the melanophores are as numerous as in wild but are smaller and of two types: one more deeply located is very small and is apparently relatively inert; and the second is more superficial, is delicately branched, and is physiologically more responsive. In Golden (which may be Fredlini of HASKINS and DRUZBA) the melanophores are large and similar to those in the wild type but only about half as numerous and are irregularly distributed. Xanthophores are well developed in this type. The double recessive is "Cream" in which the melanophores are almost entirely absent.——The third mutant is a skeletal deformity "Hunchback" which is present at birth. It is an autosomal recessive and is not linked to Blond. In a dorsoventral twist of the backbone the number of vertebrae remain constant but they are badly deformed. Chemical analysis shows an excess of calcium. The bent region is the last to calcify. Deformation appears to be due to malformation or malfunction of muscular and connective tissue elements. A second, later maturing, type of hunchback has been found, but the mode of inheritance is obscure.

GORDON, MYRON, New York Zoological Society, New York: *Frequencies of seven dominant genes in natural populations of Platypoecilus maculatus and experimental evidence of a multiple allelic series.*——On the basis of 860 Mexican *Platypoecilus maculatus* from Rio Jamapa, 3492 from Rio Papaloapam, 115 from Rio Coatzacoalcos, and 518 Guatemalan specimens from Rio Usumacinta, the distributions of frequencies of seven genes have been determined. The genes concern a series of black patterns which appear in the posterior part of the fish's body: one-spot, $P^{O}$; moon, $P^{M}$; moon complete, $P^{Mc}$; crescent, $P^{C}$; crescent complete, $P^{Cc}$; comet, $P^{Co}$; and twin-spot, $P^{T}$.——All appear in the Papaloapam populations; $P^{C}$ is missing from Coatzacoalcos, $P^{M}$ and $P^{Mc}$ are absent in Jamapa, and $P^{Mc}$, $P^{C}$, $P^{Cc}$, and $P^{T}$ are not present in Usumacinta. ——In addition to the seven single units found, there were 17 double but no triple or more complex combinations. This suggests the presence of a series of multiple alleles.——Some of the members of the Rio Jamapa and Papaloapam populations were tested genetically in the laboratory and a series of seven dominant, multiple alleles was confirmed.——When this *P. maculatus* series was tested by hybridization with *Xiphophorus hellerii*, only $P^{Co}$ was found subject to a specific modifying gene, *E*. The combination of $P^{Co}$ of *P. maculatus* and *E* of *X. hellerii* blackens all the fins and snout (Himalayan rabbit pattern). $P^{Co}$ *E* is not neoplastic, since these patterns are composed entirely of micromelanophores.

GOWEN, JOHN W., and RONALD H. NELSON, Iowa State College, Ames, Iowa: *Genetic constitution of the mother as a means of predetermining the sex of the progeny.*——Predetermination of the sex of individual progeny and the sex ratio has been possible for the female sex through the control of the genetic constitution of either the father or mother. Our own work now shows that a similar sex prediction may be made for the male sex through a knowledge of the mother's genotype. A gene or gene complex in the left arm of the third chromosome of *Drosophila melanogaster* when incorporated into the mother's genotype leads to the production of all male progeny. In some instances the progenies from such mothers may total 200 males and no females. In something over 600 matings, covering a period of ten generations from the original parents, male producing phenotypes have always occurred when expected.—— Females carrying this male producing genotype have all male progeny without regard to the males to which they are bred. The gene thus acts as a dominant. In the male the gene in the heterozygote is without phenotypic effect.

HOLLANDER, W. F., Carnegie Institution of Washington, Cold Spring Harbor, N. Y.: *A possible case of directed mutation in the pigeon.*——Male pigeons heterozygous for the dominant sex-linked "almond" color factor and its lowest recessive allele "brown" regularly show mosaic areas of the "brown" color and others with black pigmentation (wild type or other allele). Males heterozygous for "almond" and wild type show mosaic areas with black pigment but never with "brown." The same is true for "almond" females (hemizygous sex).—— Evidence presented earlier by HOLLANDER and COLE indicates that "almond" is highly mutable, rather than that somatic loss or crossing over is involved. If this

is true, the mutation process seems strongly directed by the other allele present.

HUTT, F. B., J. H. BRUCKNER, R. K. COLE and R. F. BALL, Cornell University, Ithaca, N. Y.: *A critical test of strains of fowls bred for resistance to lymphomatosis.*——Neoplasms of different kinds, mostly lymphomatosis, cause almost half of the prevailing high mortality in poultry flocks. Over a six-year period, three different strains of White Leghorns have been bred, two of which are comparatively resistant to neoplasms, the other highly susceptible. Egg production, body size, and egg size were also improved during the experiment, but resistance to neoplasms was the major consideration. More than 95 percent of the birds succumbing to neoplasms had lymphomatosis or some other form of the "avian-leukosis complex."——In 1941–42 these three strains were tested against samples from four different flocks of White Leghorns, none of which had been selected for resistance to neoplasms, though all had been improved with respect to egg production. The samples were introduced as day-old chicks and thereafter were mixed at random with chicks of the resistant and susceptible strains. The measure of susceptibility was the proportion in each line dying of neoplasms up to 500 days of age. In all four of the stocks introduced for the test, the incidence of neoplasms was higher than in the two resistant strains. In comparison with the susceptible strain, two of the introduced strains had fewer losses from neoplasms, but two had slightly more. Egg production in the two resistant strains was higher than in any of the four introduced stocks, and the production-index, or number of eggs laid per pullet housed, was much higher in the strains bred for resistance than in the others. These results show the possibility of reducing the currently disastrous losses from this disease by breeding resistant strains.

IVES, P. T., Amherst College, Amherst, Mass.: *Heterogeneity in the lethal mutation rate within a wild population of Drosophila melanogaster.*——Lethal mutation rates were observed in control and temperature shocked series in lines of wild type flies derived from each of ten second chromosomes collected five generations earlier in central Florida. Temperature shock did not significantly affect the rate in any line. Considered together with earlier data this suggests a possible difference in shock effects on the rate of lethal mutations and on the rate of visible mutations. In both control and shock series of the present study, two of the lines showed significantly higher rates than did the others. These rates were 26 lethals in 350 tests and eight in 235, respectively; while in the other eight lines together the rate was 13 in 1487. The probability of difference between each of the high lines and the group of others is greater than .99, and of difference between the two high lines is .95+. This suggests that the population is heterogeneous for genetic factors influencing the lethal mutation rate. Lethal rates were similar in eggs and sperm, in contrast to AUERBACH's data for sex-linked lethals. Preliminary tests on four of the lines with *net b cn sp* show crossing over considerably reduced in the spindle-fiber region of the highest line, an inversion in the left arm of the second high line (no crossing over observed in the left arm), and normal crossing over in two of the other lines.

JONES, D. F., Connecticut Agricultural Experiment Station, New Haven, Conn.: *Growth changes associated with chromosome aberrations.*——Paired red and dark purple areas in the endosperm of light colored maize seeds show outgrowths in less than one percent of the color alterations. In 111 cases examined so far, no deviations from normal growth accompany the loss or exchange of both *Bt* and *Pr*. Since *Bt* is close to the centromere and *Pr* near the middle, changes that include both gene markers involve a loss or exchange of almost the entire right arm of the number 5 chromosome. If the increased growth were due to the uncovering of recessive growth regulators or the accumulation of dominant growth stimulators, all or nearly all, of the color changes which include both markers would be altered in growth. Since none of them are, some thing other than normal gene action is involved. The progeny from these mosaic seeds shows no unusual deviation from normal. Growth changes of this kind are lethal in embryos but survive when surrounded and nourished by normal tissue and furnish a basis for understanding the appearance of spontaneous neoplasms in other organisms.

KALISS, N., and T. ROBERTSON, Cornell University Medical College, New York, N. Y.: *Spleen transplantation relationships among two inbred lines of mice and their $F_1$ hybrid.*——Transplantation experiments with normal tissues in inbred lines of rats, mice, and guinea pigs, and spontaneous neoplasms (breast carcinoma, leukemia) in inbred lines of mice, have led to the following generalization. Grafts from $F_1$ hybrids will grow in related $F_1$ animals, but not in either parental line. Grafts from parental lines will grow within the line of origin and in $F_1$ hybrids, but not in unrelated lines.——An exception was found by SCHWEITZER and FURTH for spontaneous leukemias arising in $F_1$ hybrids of the inbred Ak (high leukemia) and Rf (low leukemia) lines of mice. These $F_1$ leukemias behaved like leukemias of the Ak line. They could be transplanted equally well into the Ak line and related $F_1$ hybrids, but not into the Rf line. ——It was of interest to determine whether for these lines the behaviour of normal $F_1$ tissue transplants would differ from the usual pattern. Splenic fragments from sublines Ak1 and Rfe and their $F_1$ hybrids were implanted into the ventral abdominal wall, or peritoneal cavity. At least five hosts were used for each experiment. We find the same relationships hold in this case as for tissue transplantation in other animals. Normal $F_1$ spleen will not grow in the Ak1 and Rfe lines, but will grow in related $F_1$ hybrids. Ak1 and Rfe spleens will grow within the line of origin, and in the $F_1$, but not in the unrelated line. These experiments therefore demonstrate a difference in transplantation pattern between a neoplasm and a normal tissue.

KALISS, N., and M. D. SCHWEITZER, Cornell University Medical College, New York, N. Y.: *Hereditary hemorrhagic diathesis——a case of partial sex-linkage in man?*——Hereditary hemorrhagic diathesis generally is characterized by purpura and prolonged spontaneous bleeding from the nose, uterus, and mucous membranes. It may occur at all ages in both males and females. The disease is rare. It is transmitted chiefly as a dominant in heredity, but may at times behave like a recessive.——The concept of partial sex-linkage (first

worked out for human traits by HALDANE) is based upon the cytological demonstration of homology between portions of the X and Y chromosomes in mammals. Genes located in the homologous regions should be incompletely linked with sex. The statistical distribution of a partially sex-linked trait with respect to sex in the offspring would be determined by whether the factor is carried in the father's X or Y chromosome (that is, whether he inherited the trait from his mother or father). Only the progeny of affected fathers are suitable for analysis.——Our data are derived from 90 sibships (16 pedigrees). For the children of affected grandmothers (recombination class: affected sons plus normal daughters) the crossover value is 23.5 per cent (4/17). Where the grandfather was affected (recombination class: normal sons plus affected daughters) the value is 40.0 percent (16/47). The combined value is 34.1 percent. The sex ratio for all classes is 35♂:29♀ (chi-square, 0.563). These recombination values deviate in the right direction for assuming partial sex-linkage.

KAUFMANN, B. P., Carnegie Institution of Washington, Cold Spring Harbor, N. Y.: *An exceedingly complex chromosomal rearrangement.*——The number of points of breakage (and potential breakage) induced within the chromosomes of a single nucleus following irradiation of the sperm of *Drosophila melanogaster* may be much larger than was anticipated on the basis of the complexity of previously observed rearrangements. Analysis of several thousand pairs of glands had revealed a preponderance of the two- and three-break rearrangements, with only a few of the more complex, of which a single 14-break case represented the extreme. Recently, however, a rearrangement involving at least 32 breaks has been discovered. The positions of 30 of these have been determined; others, restricted to proximal heterochromatic regions, remain of uncertain location. Of the identifiable breaks, six are in the X, three in IIL, five in IIR, three in IIIL, 12 in IIIR, and one in the fourth chromosome. Aside from its great complexity, this rearrangement is of particular interest because of the accumulation of breaks in IIIR. The probability of such a distribution on a chance basis alone is very small. In the light of this information, other data concerning break distribution in complex rearrangements have been reexamined, and these likewise suggest the same non-random distribution. Many implications are apparent and recommend a new appraisal of the factors involved in the production of these complex rearrangements. The problem will be considered in detail in another publication.

LAMOREUX, W. F., and F. B. HUTT, Cornell University, Ithaca, N. Y.: *Genetic resistance to deficiency of riboflavin in the chick.*——Strains of White Leghorns relatively resistant or susceptible to a deficiency of riboflavin have been developed. Resistance was measured by the ability to survive and to gain in body weight during the first few weeks after hatching while the chicks received a diet deficient in riboflavin, but adequate with respect to all other known nutritive requirements. The records of the individual, its siblings, its half-siblings, and of its progeny, if any, have been considered in selecting birds for breeding.——In the fifth generation 191 chicks in the resistant strain made

an average gain in weight of 62 grams to five weeks of age. This gain was 36 percent greater than that attained by 50 chicks of the susceptible strain, and 11 percent greater than that among 39 control chicks. During the same period only 9 percent of the chicks from the resistant strain died, whereas 29 percent of those from the susceptible strain, and 36 percent of the controls were unable to survive on the experimental diet. That females are more resistant to a deficiency of riboflavin than are males was shown by the fact that among chicks from the resistant strain 13 percent of the males, but only 6 percent of the females, died while receiving the deficient diet.

LEBEDEFF, G. A., University of Puerto Rico, Agricultural Experiment Station, Rio Piedras, P. R.: *Inheritance of hard-seed production in common beans (Phaseolus vulgaris).*——The seeds of 36 selections of common white beans were dried over $CaCl_2$ to ten different moisture contents, ranging from 14.11 to 5.59 percent. With each reduction in the seed moisture content the percentage of hard seeds increased while the rates of their softening slowed down. Individual selections differ greatly in their response to drying of seeds, the differences in some instances were large and conspicuous, in other instances they were small but persistent. Some selections developed greater percentages of hard seeds than others, and in some selections the hard seeds softened readily when germinated, while in others they remained hard for a long time.——Statistically very significant correlations were found between the percentages of hard seeds in all 36 selections on any day of any two germination tests, irrespective of the season in which they were grown, age of the seeds, their viability, and the seed moisture content. This indicates that the ability of the individual selections to respond in a certain way in regard to hard seed production is inherited.——The hard seed production of the $F_1$ seed generation in several cases was intermediate between that of the parental selections; in one cross it was intermediate only during the first seven days of germination test; and in another cross the rate of softening of the hard seeds of the $F_1$ seed generation was considerably lower than in either of the parental selections.

LERNER, I. MICHAEL, University of California, Berkeley, Calif.: *The failure of selection to modify shank-growth ratios of the domestic fowl.*——Previous investigation has established the existence of a uniform pattern of growth of the tarsometatarsus relative to body weight in different breeds of chickens. However, some variation between individuals was observed. This suggested the possibility that within the limits of the specific growth-pattern, genetic variation in growth-ratios may still be present. Hence, progeny testing of birds originating from a Leghorn×Rock cross and selection for respectively high and low shank-growth ratios ($\alpha$) were inaugurated. The $\alpha$ values for individuals were obtained from least squares solutions of the equation——shank length $= b \times$ body weight$^{\alpha}$, using five weekly weighings and measurements (four to eight weeks of age). At the end of four generations of progeny-test selection involving 320 offspring the high $\alpha$ and the low $\alpha$ lines did not differ from each other. It may then be concluded that the genetic variability of the relative growth pat-

tern in the stock used is either non-existent or is so low as to preclude its demonstration by the methods of selection in opposite directions employed here. This supports the hypothesis previously advanced that the stocks of chickens investigated belong to the same heterauxetic tribe.

LINDEGREN, CARL C., and GERTRUDE LINDEGREN, Washington University and Anheuser-Busch, Inc., St. Louis, Mo.: *Legitimate and illegitimate mating in Saccharomyces cerevisiae.*——WINGE and LAUSTSEN showed that in *Saccharomyces cerevisiae* each ascus contains four spores which produce haploid gametes that mate freely among themselves. They found that matings between gametes originating from the same single ascospore produce zygotes of somewhat smaller size, and cultures of this origin produced ascospores of very low viability. They concluded that degeneration resulted from homozygosis of dysgenic hereditary factors. We have reached a different conclusion, for in our experiments degeneration in spore viability did not occur after five generations of inbreeding. Each generation was originated by cross-breeding two gametes from different ascospores, but both ascosopores had developed in the same ascus. We have concluded that segregation of different mating type genes occurs in the ascus and that each ascus contains ascospores of different mating types. Matings between two individuals which properly complement each other as to mating type are necessary to give fully vigorous fertile zygotes capable of producing four-spored asci containing viable ascospores. An illegitimate mating between haploid cells of the same mating type produces a degenerate form. Degeneration is specific and arises primarily from homozygosis of mating type alleles rather than homozygosis of other random dysgenic factors. Illegitimate matings produce diploid zygotes homozygous for mating type alleles which differ from the normal heterozygous diploid form in the following characters: (1) Vegetative cells are usually smaller and round rather than large and ellipsoidal. (2) Ascospore production is markedly diminished. (3) Ascospores are generally non-viable. (4) Ascospores are generally smaller in size. (5) Asci are one- and two-spored in contrast to the heterozygous cultures which contain numerous four-spored asci. (6) The total amount of growth is generally smaller.

LINDSTROM, E. W., Iowa State College, Ames, Iowa: *Experimental data on the problem of dominance in quantitative character inheritance in maize and tomatoes.*——Using paired, randomized plantings of $F_1$-$F_2$ and of $F_2$-$F_3$ generations, the mean values of maize earliness (tasseling and silking dates) and of tomato fruit size were obtained. In contrast to the usual decrease of means with successive inbreedings, these characters showed an increase. In four crosses of maize inbreds the $F_2$ mean was significantly 'larger' (later) than the $F_1$; and in another cross the $F_3$ was later than the $F_2$ mean. In one tomato cross (Yellow Cherry × King Humbert) the $F_2$ mean fruit weight was larger than the $F_1$ mean. These results may be explained on the hypothesis of a dominance in genes for small size, the case in maize being interpreted as dominance in fewer days to flowering. For genes with no dominance and with either additive or

geometric interaction no significant change in mean values would result from successive generations of inbreeding. Certain forms of epistasis or other factorial interactions are not ruled out as partial explanations of such results, but until these interactions are actually proven to exist, the hypothesis of dominance, whether dominance for small or for large size, would seem to be effective in explanation of quantitative character inheritance. The universality of dominance for large size in characters showing heterosis is reflected in the usual decline in mean size of $F_2$ and $F_3$. Data on this point involving ear characters of maize are included.

MARENGO, N. P., New York University, New York, N. Y.: *The effect of the gene abdomen rotatum on the development of Drosophila melanogaster.*——Observations on the development of normal and genetically rotated individuals demonstrate the existence of certain puparium characters offering reliable means of identifying genetically rotated prepupae many hours before actual rotation occurs. These are (1) the persistence of marked segmental constrictions in the puparium which are lost in the hardening of the normal puparium; (2) a deeply marked constriction near the posterior end of the puparium just anterior to the spiracles; (3) a marked bulging of the lateral abdominal wall between the attachments of the lateral dorso-ventral muscles in the segments just anterior to the posterior constriction; (4) transverse ridges of a distinctly abnormal character. All puparium abnormalities described are symmetrical in nature and appear before actual rotation, which is the first indication of any asymmetry in development.——Rotation was observed to coincide with the termination of the prepupal period and the movements attendant on prepupation. Torsion of the internal abdominal organs in the pupae is in the same direction and of the same degree as rotation of the external pupal segments. ——Evidence suggests that the persisting larval muscles in the abdominal region are responsible for the prepuparial abnormalities and may also be in the same way involved in the development of the rotation. The adult muscles play no part in the development of the rotation.

MIRSKY, A. E., and A. W. POLLISTER, Hospital of the Rockefeller Institute and Columbia University, New York: *Nucleoproteins of chromosomes.*——We have prepared nucleohistones from nuclei of so many different cells, plant as well as animal, that histone (defined by its basic isoelectric point and by presence of tyrosine and absence of tryptophane) may now be considered as nearly a universal nuclear constituent as desoxyribose nucleic acid. All desoxyribose nucleic acids examined contain the purine and pyrimidine nucleosides in equimolar quantities—a definite restriction on possible variations in these nucleic acids. Variations in the histones of different species can be recognized by differences in amino acid content. A clear and decisive answer can now be given to the question of whether there are also variations in chemical composition of the chromosomes of different tissues of the same organism. Comparison of nucleoproteins prepared from chromosomes of mature sperm, immature gonads, erythrocytes and liver of *Salmo salar* and *Salmo fario* show definite differences

in the nature of the basic proteins. Nucleoprotein of mature sperm contains protamine (defined by its extremely high nitrogen content, small molecular size and absence of both tyrosine and tryptophane) and no histone, whereas nucleoproteins of erythrocytes and liver contain histone and no protamine. In nucleoproteins prepared from the immature gonad both histone and protamine are present. When comparing nucleoproteins prepared from erythrocytes and mature sperm the problem of cytoplasmic impurities does not arise, because the preparation is made from isolated chromosomes of the erythrocytes and because in the sperm used well over 90 percent of the cell substance is in the sperm head. The transition from histone to protamine that occurs during spermatogenesis of the salmon shows that if these basic proteins are indeed part of the germinal material, the relatively simple constitution of the protamine suffices to transmit the properties of the gene.

MULLER, H. J., Amherst College, Amherst, Mass.: *Mutation rate dependent on the size of the X chromosome.*——A gene *m*, causing higher mutation rate than its allele, will tend to be selectively eliminated only to the extent that the resulting abnormals—that is, descendants phenotypically manifesting mutations induced by *m*—also receive *m* itself. Every additional generation, after $F_1$, intervening between origination and manifestation of the mutations so induced, halves (approximately) the chance of the abnormal individual's receiving *m* and correspondingly reduces the selection against *m*. Dominant mutants, being manifest in $F_1$, are relatively efficient (dominant complete lethals 100 percent efficient) in working for elimination of *m*, but there seem far fewer dominant than recessive gene mutations attaining a given high or moderate grade of inviability or infertility. Autosomal recessives, however, usually manifest themselves so long after their origination that their effectiveness in causing elimination of *m* is negligible for present purposes. But sex-linked recessives are comparable with autosomal dominants in promptness of manifestation, and where (as probably in *Drosophila melanogaster*) decidedly harmful sex-linked mutations considerably outnumber corresponding autosomal dominants, the former must play the chief role in the elimination of *m*, and thus in the determination of the equilibrium mutation rate of the population. Since elimination of *m* genes is faster, the greater the number of sex-linked mutations they induce, other things being equal, those species which have more of their genes sex-linked will have had more effective selection against high mutation-rate genes and will have attained a correspondingly lower "normal" mutation rate. Formulae for the relations (giving almost inverse proportionality of mutation rate to size of X) are presented. Preliminary reported evidence of other authors indicates *pseudoobscura*, with its double-size X (active regions), to have about half the *melanogaster* mutation rate. Haploid sexes or generations would lead to minimal mutation rates.

MYERS, W. M., U. S. Regional Pasture Research Laboratory, State College, Pa.: *The effects of inbreeding upon meiotic irregularity in plants of Dactylis glomerata.*——In *Dactylis glomerata*, a naturally occurring autotetraploid

(x=7), chromosomal association and behavior during meiosis was studied, using five to 20 inbred ($I_1$) plants from the progenies of eight clones known to differ significantly in meiosis. The plants in each progeny segregated for quadrivalent frequency per sporocyte. In each of six progenies, the average number of quadrivalents for the inbred plants was nearly identical with the value for their parents, values for progeny and parent respectively being 4.08–4.09, 4.04–4.06, 3.99–3.92, 3.98–3.95, 3.80–3.79, and 3.50–3.50. In two progenies, quadrivalent frequency increased significantly with inbreeding: 3.50–3.04 and 3.66–3.00.——In all families the inbreds averaged higher than their parents in percentage of metaphase I with univalents (6–3, 10–5, 12–4, 12–4, 13–6, 30–14, 33–9, 42–24), anaphase I with lagging univalents (9–4, 12–7, 17–5, 22–7, 25–8, 43–26, 46–29, and 58–28), and quartets with micronuclei (13–4, 13–5, 15–4, 21–11, 38–13, 46–22, 49–35, and 52–28). The frequency of half-chiasmata per chromosome was obtained for two families. In one with 20 $I_1$ plants, the averages of $I_1$ and parent, respectively, were 1.71 and 1.78. This progeny was identical with its parent in average quadrivalent frequency (3.50). In the other progeny with six $I_1$ plants, the average chiasma frequency of progeny and parent was 1.82 and 1.77, while the number of quadrivalents was 3.66 and 3.00.

NEEL, JAMES V., Dartmouth College, Hanover, N. H., and Columbia University, New York, N. Y.: *Factor interaction and the relation of chaetae number to size in Drosophila melanogaster males homozygous for Hw, h, pyd, and the combinations of these factors.*——Wild type, *se h, pyd, y Hw, y Hw;pyd, y Hw;se h, se h pyd*, and *y Hw;se h pyd D. melanogaster* males were studied as to the relation between fly size (measured by femur length) and the number of dorsocentral bristles, number of teeth in the right sex-comb, number of scutellar bristles, number of scutellar hairs, and number of hairs on the right second longitudinal wing vein. In each case the regression of chaetae number on fly size was adequately represented by a straight line on an arithmetic plot. The last three characteristics mentioned showed reasonable agreement with the hypothesis that the ratio of percentage increase in the number of supernumerary chaetae to percentage increase in femur length was closely similar if not actually identical in all genotypes, while the first two characteristics showed no such constancy.——In previous work on the interaction of these factors, an accurate evaluation of gene effects had been impossible in view of the mean size differences between the various strains under favorable food conditions. The derivation of the regression equations made it possible to estimate chaetae number at a size common to all flies. The exact quantitative picture of factor interaction was then found to differ according to the kind of chaetae under observation. With respect to the last three characteristics mentioned above the data can be interpreted as indicating that primary genetic effects are for the most part additive, but that the subsequent relation of genetic effect to phenotype is different in each case. In the other two systems less regular effects, thus far not fitted into any relatively simple scheme, were encountered.

OLIVER, C. P., and R. C. ANDERSON, University of Minnesota, Minneapolis: *Infertility due to sperm non-motility in a female of Drosophila melanogaster.*—— Glossy females, an allele of lozenge, are very infertile and lack spermathecae and parovaria; the ventral receptacles seem normal. Glossy females were mated to normal males, then isolated for periods of time at 24 hour intervals and dissected, and the ventral receptacles were examined for sperm. Normal females were treated similarly. Glossy females showed a smaller average initial number of stored sperm than did the controls. Sperm retained their viability in control females, as indicated by sperm movement, over the eleven-day period, the percentage of females with motile sperm in the ventral receptacle ranging from 93 to 100 through the test period. Glossy females showed a gradual daily decrease in the percentage of females with motile sperm, ranging from 100 on the first day to 16 percent on the sixth day and 0 by the seventh. The results agree with the known breeding data in which glossy females produce approximately ten offspring per breeding female, and they occur within the first two days after copulation, although eggs are produced in abundance throughout the test period. Sperm become inviable or non-motile in the ventral receptacle of the females within at least 48 hours. Glossy females which were mated a second time, on the eleventh day produced offspring again; and in contrast to controls, the new offspring were only from the second sperm supply.

PADDOCK, ELTON F., Ohio State University, Columbus, Ohio: *The backcross transmission of an inversion in an interspecific hybrid of Solanum.*——*Solanum Douglasii* Dunal and *S. nodiflorum* Jacq. are diploids ($2n = 24$) of the *S. nigrum* polyploid complex. Meiosis in their hybrid when *Douglasii* is female is nearly regular, having a mean of 11.8 bivalents per P.M.C. (126 cells analyzed). Bridges with corresponding fragments seen at AI and AII in P.M.C. indicate heterozygosity for at least one inversion. No such configurations were found in P.M.C. of 14 plants from pollinating the hybrid by *Douglasii*. Of 15 plants from pollination by *nodiflorum*, 11 did show such configurations, three showed bridges but not confirmatory fragments, and one showed neither bridges nor fragments. To explain these facts, it is hypothesized that the *nodiflorum* member of the chromosome pair involving the inversion bears a gene which reacts with the *Douglasii* cytoplasm of ovules which receive it in the hybrid to prevent their functioning. Crossing over between this gene and the inversion could account for the absence of the inversion in heterozygous state in the exceptional *nodiflorum* backcross plant. The occurrence of bridges and fragments at AII is evidence that crossing over may occur proximally to the inversion with disparate relationship to chiasmata within the inversion. A configuration showing two bridges and two fragments at AII (one of each between each set of separating nuclei) constituted evidence that at least three simultaneous chiasmata may form in a single pair of chromosomes. The maximum observable number per normal bivalent at diakinesis or later stages is two due to early complete terminalization in these species.

POLLISTER, A. W., and A. E. MIRSKY, Columbia University, and The Hospital of the Rockefeller Institute for Medical Research, New York, N. Y.: *The isolation of chromosomes from resting nuclei.*——From the blood cells of salmon, carp, and fowl the chromosomes can be isolated by the following method. The cells are washed repeatedly with isotonic sodium chloride. The washed cells in suspension in the saline are then finely minced in a high speed mixer (Waring Blendor), and the fragments are sedimented by centrifuging. After several repetitions of this procedure, the cells and nuclei are all broken up. The suspension then consists almost entirely of short threads, fairly uniform in diameter, which are several times as long as metaphase chromosomes. The volume of one of the longest threads from salmon blood is not more than one two-hundredth of the volume of the resting nucleus of the salmon erythrocyte. That these threads are the chromosomes is shown by their size (the diploid number would only partially fill the resting nucleus) and by their strong positive Feulgen reaction. The chromosomes dissolve completely in 1.0 molar sodium chloride; and from the viscous solution a typical desoxyribose nucleohistone can be precipitated by dilution. Analysis of the complete chromosomes indicates that they consist almost entirely of desoxyribose nucleohistone, with no more than a slight trace of ribose nucleic acid, if any.

POULSON, D. F., Yale University, New Haven, Conn.: *Origin and early development of the nervous system in Drosophila melanogaster in normal and Notch-deficient zygotes.*——A full analysis of the developmental abnormalities characteristic of deficiencies for the facet locus (typical Notches) has necessitated a study of the origin and development of the normal nervous system of Drosophila.——Eggs from the Oregon R and Notch strains, incubated at 25°C, were fixed at intervals, sectioned and silver impregnated by a modification of the Bodian method. Other sections were stained with iron-hematoxylin.—— First indications ($4\frac{1}{2}$ hours) of the normal nervous system are the neuroblasts which appear lateral to the ventral midline and in the region of the brain and come to lie between the ectoderm and mesoderm. These undergo mitoses and become fairly numerous. After 6 hours (from fertilization) these undergo unequal mitoses producing small nerve cells and larger cells which continue unequal division. In 8–10 hour embryos the ventral nervous system consists largely of these smaller cells and extends the length of the embryo. When the embryo undergoes shortening after 10 hours these become closely compacted, and regions of nerve fiber outgrowth become apparent. Segmental nerves begin their outgrowth and are more easily seen at later stages when the ventral nervous system becomes still more condensed. In embryos older than 12 hours few mitoses are observed, and these are principally confined to the brain. This indicates that the larval nervous system, like other larval structures, undergoes much of its visible differentiation after mitosis has ceased. In 15 hour embryos nerve fibers and paths are clearly traceable. In the deficient embryos there is hypertrophy of the entire nervous system, nerve fibers are slower in differentiating, and the parts of the nervous system are irregularly connected. The cells in normal and abnormal nervous systems are of the same size.

POWER, MAXWELL E., Osborn Zoological Laboratory, Yale University, New Haven, Conn.: *Neurological action of genes which reduce eye size in Drosophila.*——It has formerly been demonstrated that the optic glomeruli of *Drosophila melanogaster* undergo a volumetrical hypoplasia which is proportional to the genetical induced reduction in the size of the compound eye. Further study of the Bar alleles and eyeless-2 in microscopic sections has shown that the volume of the glomeruli is correlated only with the facet number of the adjacent side. The ommatidia present on one side have no detectable effect upon the optic glomeruli of the opposite side, the ommatidia-glomerulus relationship being autonomous on each side. In cases where fibrillar attachment is not established between ommatidia and the brain the optic glomeruli are histologically like and volumetrically not statistically different from those of completely eyeless flies. Thus it is suggested that the hypoplasia of the gomerulus is not a primary action of the genetic factors which reduce facet numbers but rather a secondary result of the ingrowth of a smaller number of centripetal fibers from the genetically reduced peripheral eye field.

RUSSELL, W. LAWSON, and EARL L. GREEN, Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Me., and Ohio State University, Columbus, Ohio: *A skeletal difference between reciprocal $F_1$ hybrids from a cross of two inbred strains of mice.*——Variation in number of lumbar vertebrae in the C57 black and C3H strains of mice and their reciprocal hybrids is defined by the percentage having five lumbar vertebrae—the remaining animals showing either six, or a tendency toward that condition as indicated by asymmetry at the lumbosacral border. Three hundred thirty-five male and 303 female hybrids, obtained in 89 litters from 13 C57 black female×C3H male matings, showed respectively 33.4 and 24.1 percent with five lumbar vertebrae. The reciprocal hybrid gave 68.1 percent in males and 45.8 percent in females, out of respectively 182 and 166 animals in 60 litters from 13 matings. The difference between the reciprocal hybrids is statistically significant in each sex even when allowance is made for possible littermate correlations. In the parental strains, 498 C57 black animals showed 1.4 percent, and 980 C3H animals 96.9 percent with five lumbar vertebrae. The reciprocal hybrid difference therefore, is, in the maternal direction. A total of 1278 $F_2$ animals showed no significant difference between the progeny of the two $F_1$ reciprocals, indicating that in neither sex can the difference between reciprocals be attributed to genetic factors. It may therefore be concluded that the difference is due either to cytoplasmic inheritance or, more probably, to a uterine influence.

RUSSELL, ELIZABETH S., and W. LAWSON RUSSELL, Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Me.: *A quantitative histological study of genic effects on coat color in the house mouse.*——Histological study of size, shape, quality and intensity of color, number, and distribution of hair pigment granules in a wide variety of genotypes, involving $A^y$, $A^w$, *a*, *B*, *b*, *P*, *p*, *D*, *d*, *C*, $c^{ch}$, $c^a$, and *c*, has provided new information on the action of these genes.——In *aa* combinations, and non-yellow regions of $A^wA^w$, the granules vary greatly

in size, shape, color, and number. Substituting *bb* for *BB* always changes granules from black-sepia to brown, but rarely affects number. Substituting *pp* for *PP* always produces an arrangement characterized by many tiny shreds of pigment with a few flocculent masses. The chief effect of substituting *dd* for *DD* is reduction in number of granules and partial clumping. Substituting $c^{ch}c^{ch}$ for *CC* in *aaBBDDPP* reduces size, but not number, of granules, and has no effect in *aabbDDPP*. All other *C*-series substitutions studied affect granule number, while some apparently affect intensity, but never quality, of granule color.——In $A^{v}a$ combinations, and yellow regions of $A^{w}A^{w}$, the granules are bright yellow spheres of constant size, varying only in number. The gene *d* and the *C*-series, which affect number of granules in *aa* combinations, have a marked effect on $A^{v}a$ genotypes, while *b* and *p*, which on an *aa* background change other granule characteristics much more than number, have little effect on $A^{v}a$ combinations.——In all combinations, variation in distribution of granules seems to be associated with the quantity of pigment produced irrespective of the particular genes concerned.

SAWIN, P. B., and D. GLICK, Brown University, Providence, R. I., and Newark Beth Israel Hospital, Newark, N. J.: *Inheritance of "atropinesterase," a blood enzyme of the rabbit.*——Early studies of the effect of atropine on the animal body showed that certain species, including man, monkey, cat, and dog, are highly sensitive to the drug, while others, such as the rat, guinea pig, rabbit, goat, and pig, are relatively insensitive. Examination of the serums of 540 rabbits belonging to five unrelated families and crosses between them shows not only that the blood of some rabbits can destroy atropine while that of others cannot, but that the ability to destroy it is transmitted in a typical mendelian manner. Enzyme activity was determined by the manometric method employing the Warburg apparatus as has been described elsewhere. Blood serums of animals of the proper matings at three months of age or older were taken in the laboratory at Providence and sent by mail to Newark to be examined within three days. Rabbits which possess this enzyme and are thus able to hydrolyze atropine inherit the peculiarity in a gene (*As*) borne in the same chromosome as the gene (*E*) for the extension of black pigment in the coat. The gene (*As*) is incompletely dominant, homozygotes producing the enzyme more effectively than heterozygotes. The enzyme is not present at birth, appears first at about one month of age, and tends to be the greater in females. The present study illustrates the manner in which inheritance of other biochemical variations occurring in the larger laboratory animals could be approached effectively and economically through cooperation.

SCOTT, J. P., Wabash College, Crawfordsville, Ind.: *Differences in the social organization of mice caused by differences in social behavior (fighting of males).* ——When kept in similar environments, males of the C-57 and C3H inbred strains (originally from the Jackson Laboratory) show differences in fighting behavior. Either strain may be taught to fight or not to fight. With equal training, the C-57 males become more active fighters or are more easily in-

hibited. Small amounts of repressive training may leave the C-57 males inhibited and the C3H not.——When two males of one strain are placed together the following types of social organization are seen, both heredity and training being important indirect factors: (1) C-57, trained to fight—one male dominant, and loser avoids winner, (2) C-57, inhibited—no dominance, (3) C3H, trained to fight—dominance, but loser does not avoid winner, (4) C3H, inhibited—no dominance. These results help to confirm the theory that social organization is determined by the social behavior of the individuals concerned, and artificial societies of mice may assist in the solution of certain simple human problems.

SINGLETON, W. R., Connecticut Agricultural Experiment Station, New Haven, Conn.: *Breeding behavior of C30 a diminutive P39 mutant whose hybrids show increased vigor.*——Purdue 39, the most widely used sweet corn inbred, mutated to a semi-dwarf form, C30. This inbred is entirely normal and similar to Purdue 39 in every respect except smaller. The average height of all P39 plants in 1942 was 72 inches, while C30 plants were only 42 inches high. The ear size is similarly reduced. C30×P39 shows complete dominance of the P39 growth habit. Such plants when selfed produce 25 percent of C30 type plants. The $F_1$ hybrid backcrossed to C30 produces 50 percent of each type, showing clearly a segregation due to a one gene difference. When C30 and P39 are each crossed to a common inbred C13, the C30×C13 crosses yield in some cases significantly more than the P39×C13 hybrids. The difference is more pronounced when P39 and C30 are crossed by C15. Here seems to be a truly heterotic factor giving increased vigor in hybrids although the inbred itself is much reduced in size.

SMITH, HAROLD H., Bureau of Plant Industry, Beltsville, Md.: *Effects of different proportions of specific chromosomal complements on size in Nicotiana.*——From crosses among certain doubled or undoubled chromosomal forms of *Nicotiana Langsdorffii* Weinm. (n=9), *N. Sanderae* W. Wats. (n=9), and the $F_1$ *Langsdorffii*×*Sanderae* the following polyploid combinations were obtained: LLLS, LLS, LSS, and LSSS. L represents a haploid complement of *N. Langsdorffii* (small-flowered species) and S a haploid complement of *N. Sanderae* (large-flowered species). The size of corollas in the intermediate types was directly proportional, on a logarithmic scale, to the relative amounts of *Langsdorffii* and *Sanderae* genoms present. When the data were arranged in series so that two complements of either species were added, one at a time, to different genetic backgrounds, it was apparent that the addition of the second complement caused a smaller change and rate of change in corolla size than the addition of the first. The effect of total chromosome number was observed by comparing the following 18 and corresponding 36-chromosome types: LL vs. LLLL, LL×LS vs. LLLS, LS vs. LLSS, LS×SS vs. LSSS, and SS vs. SSSS. In each type the length of the tubular part of the corolla was increased by the double number of chromosomes, but the limb measurement was not appreciably affected. The effects of four different single extra chromosomes from *N.*

*Langsdorffii* on the background of the $F_1$ were studied. It was found that two of the extra chromosomes each caused a decrease in the size of all regions of the corolla, whereas the other two caused an increase in the size of one region and decrease in another.

SONNEBORN, T. M., Indiana University, Bloomington, Ind.: *Development and inheritance of serological characters in variety 1 of Paramecium aurelia.*—— Dilute antiserum from a rabbit injected with stock P of variety 1 of *Paramecium aurelia* paralyses and kills this stock, but not stock 60. The principal antigen that differentiates stock P from stock 60 is determined by a single dominant gene. When the heterozygous hybrid (P×60) is selfed or crossed to the recessive, the antigen can still be detected in the recessive progeny for four to eight fissions ("cytoplasmic lag"); thereafter it cannot be detected. When the dominant gene is introduced into recessives, the antigen cannot be detected in the heterozygotes for several fissions; then, over a period of many fissions, the antigen-antibody reaction gradually increases in intensity until it reaches the level normal for the stock P.—— Dilute antiserum against the 100 percent homozygous stock 60 paralyses and kills nearly all members of this stock, but in some clones individuals arise that are unaffected by this antiserum. The serum-fast condition is lost within a few fissions unless the serum-fast animals are exposed to the antiserum. After such treatment, the serum-fast condition persists during vegetative reproduction. Many lines are still serum-fast after more than 275 cell generations in antiserum-free culture medium, but other lines lose their serum-fastness after fewer fissions. It is always lost after either cross or self fertilization within nine fissions. These results agree with some of those in the literature under the name of Dauermodifikationen. They appear to throw some light on the genetic nature of some of the well-known but puzzling persistent changes of virulence in pathogenic organisms.

STADLER, L. J., and SEYMOUR FOGEL, U. S. Department of Agriculture and University of Missouri, Columbia, Missouri: *Gene variability in maize. I. Some alleles of R ($R^r$ series).*——The gene *R* affects the occurrence and extent of anthocyanin pigmentation. Known alleles include forms with colored seed and plant (here designated $R^r$), colored seed and colorless plant ($R^g$), colorless seed and colored plant ($r^r$), and colorless seed and plant ($r^g$). Previous experiments have shown that in spontaneous mutation of $R^r$, mutations affecting seed and plant color occur independently.——The present study is a comparison of alleles of the $R^r$ class from 19 relatively unrelated individual plants (chiefly from strains grown by different Indian tribes), taken as a sample to indicate the variability in the naturally occurring forms of this gene. Twenty-two stocks were included in the comparisons, since in three cases a single foundation plant yielded two distinguishable $R^r$'s.——The alleles were compared in backcross segregations with $r^{ch}$ (maximal plant color) and $r^g$ (no plant color), and in matings of the type $R^xR^y \times r^g$. In effect upon plant color, the alleles differ (1) in specific regions affected and (2) in intensity of pigmentation in certain regions. The series is non-linear—that is, in various cases one allele

excels another in pigmentation of certain regions, while the reverse is true of pigmentation of other regions. In such cases compounds show in each region pigmentation similar to that of the more pigmented parent.——Additional criteria applied in comparing the alleles were (1) reaction to a modifier inducing variegation of *R*-determined pigmentation, (2) aleurone color in homozygous and heterozygous endosperms, and (3) frequency of spontaneous mutation. The results indicate that few if any of the 22 $R^r$'s studied are identical.

STADLER, L. J., and HERSCHEL ROMAN, U. S. Department of Agriculture and University of Missouri, Columbia, Missouri and University of Washington, Seattle, Wash.: *The genetic nature of X-ray and ultraviolet induced mutations affecting the gene A in maize.*——Among a large number of alterations affecting *A* induced by X-ray treatment, and a smaller number induced by ultraviolet treatment, those most nearly approaching the typical genetic behavior of gene mutations were selected for detailed study and comparison. With X-rays, only a very small proportion of *A*-alterations are haplo-viable, and only two cases were found with normally developed pollen. The X-ray mutants studied included these two (*a*-X1 and *a*-X2) and a defective pollen type, *a*-X3, which is haplo-viable in the female gametophyte. The ultraviolet mutants studied, all of which produce normal pollen, include *a*-U1, *a*-U2 and *a*-U3, with phenotypic effects similar to *a*, and $A^{lt}$, with phenotypic effects intermediate between *A* and *a*.——All three X-ray mutants show lowered haplo-viability, as indicated by transmission of the mutant *a* (general designation *a**) through ♂ and ♀ germ cells of *A a** individuals. Transmission of *a*-X1 is lowered thru ♂ gametophytes and normal thru ♀; *a*-X2 much lowered thru ♂, somewhat lowered thru ♀; and *a*-X3 lacking thru ♂, much lowered thru ♀. Both *a*-X1 and *a*-X2 are lethal when homozygous, and compounds of the X-ray mutants in all three possible combinations are lethal. Crossing over in *A a** plants is approximately normal with *a*-X1, lowered with *a*-X2, and much lowered with *a*-X3. In all four ultraviolet mutants, ♂ and ♀ transmission is normal, homozygotes and compounds are viable and are normal in development, and crossing over is normal.——By the use of an unstable duplication covering the *A* region, homozygotes and compounds of the various mutants were produced and sectors resulting from loss and alteration of the duplication segment studied. These studies show that all three X-ray mutants involve loss of the effects not only of *A* but of additional genes affecting chlorophyll development and cell viability. All four ultraviolet mutants are free from these associated effects.

STEINBERG, A. G., and F. C. FRASER, McGill University, Montreal, Que.: *The interaction of rhino with Naked and hairless.*——Rhino-hairless ($hr^{rh}/hr$) mice lose their hair in the same way as hairless, but show wrinkling like but not as extreme as that of rhino. In heterozygous Naked; rhino-hairless ($N/n$; $hr^{rh}/hr$) mice the first appearance of the hair coat is delayed. The coat is short and rough. Depilation of the body is typically Naked in pattern but the feet show typically hairless depilation. Wrinkling is characteristically rhino.

The skin remains pigmented (70 days to date). *N/N; hr$^{rh}$/hr* mice lack vibrissae and hair at all times. Wrinkling is typically rhino but exaggerated. The nails are soft and thin but show a definite tendency to elongation and spiralization as in rhino. MR. FRASER'S studies of the histology of *hr$^{rh}$*, *hr*, *N* and their combinations indicate that rhino skin is similar to hairless in that it shows the same irregular shortening of the follicles before depilation; however, rhino forms a greater number of hair canal cysts. This is probably the reason for the increased wrinkling shown by rhino mice. Preliminary observations indicate that there is a widening of the hair canals in both rhino and hairless mice immediately preceding hair loss. It is likely that this is the immediate cause of depilation. The interactions of rhino with hairless and Naked may be explained as follows: Hairless and rhino affect the same chain of reactions, but rhino more so than hairless. In Naked, weaknesses in the hair shaft result in a bending and buckling of the hairs during their eruption. The consequent irritation of the hair canal accentuates the tendency toward cyst formation evident in hairless and rhino mice.

STEINBERG, A. G., and F. C. FRASER, McGill University, Montreal, Que.: *Marcelled, a new recessive mutation affecting the coat of the house mouse.*——Marcelled (suggested symbol *ma*) is an autosomal recessive mutation whose most obvious phenotypic expression is a waviness of the coat in young mice. The wave is tighter than that of *wa-1* or *wa-2*, and the individual hairs are shorter and more irregular than those of *wa-1* or *wa-2*. The vibrissae of *ma* mice are short and very irregular, much more so than those of *wa-1* or *wa-2*. As yet no comparisons between *ma* and *Ca* have been made. The irregularity of the vibrissae permits classification of *ma* at birth. In the adult the coat loses its waved appearance but remains short and rough. Crosses with *wa-1* and *wa-2* show that *ma* is not allelic to or linked with either. In the $F_2$ of *ma*×*wa-1* four distinct phenotypes result: wild type, *wa-2*, *ma*, and *wa-2;ma*, the last named being the most extreme waved. Work in progress indicates that the same is true of the $F_2$ between *wa-1* and *ma*. This would suggest that *ma* affects the coat through a different mode of action than either *wa-1* or *wa-2*. Work in progress indicates that *wa-1* and *wa-2* affect the same reaction system since the $F_1$ of *wa-1*×*wa-2* is not wild type but very slightly waved. The interaction between hairless and marcelled is similar to that described by DAVID for hairless and waved. Linkage tests made to date indicate that *ma* is not linked to *A*, *B*, *hls*, or *wa-2*. Further linkage tests are planned. Both sexes are fully fertile and their viability is excellent.

STERN, CURT, University of Rochester, Rochester, N. Y.: *Cumulative and competitive action of alleles and their bearing on the position effect.*——In Drosophila the fourth chromosome gene cubitus interruptus (*ci*) is recessive to wild type (+) at 25° and slightly dominant at 14°. At both temperatures the effect of two doses of *ci* is more normal than that of one and less than that of three. Thus, *ci* contributes toward normality. The normal allele alone, in single dose, assures normality at 25° but not at 14°, where a slight *ci* effect appears. At

14° the heterozygote +/*ci* deviates more from normality than flies carrying the +-allele alone: the cumulative effect due to "addition" of *ci* to a genotype containing *ci* only, is reversed into an antagonistic effect if *ci* is added to the +-genotype. Possession of both positive and negative properties of the *ci*-allele may be interpreted, following WRIGHT, in terms of (1) power of the gene to combine with a limited cellular substrate (S) and (2) efficiency of the gene in converting S into a product involved in formation of normality. The *ci*-allele appears lower in both combining power and efficiency than the +-allele. ——Position effects of the +-allele (DUBININ) show decrease of dominance of + without a change in expression of the homo- or hemizygous allele. Position effects of the *ci*-allele (T (*ci*); DR. G. HEIDENTHAL and author) demonstrate increase of dominance of *ci T* (*ci*)/+ and decrease of cumulative action in T (*ci*)/*ci*. The effects become intelligible if considered as due to competition for S which is decreased in these translocations relative to the combining power of the alleles.

STRAUS, F. S., and J. W. GOWEN, Iowa State College, Ames, Iowa: ***Heterosis: its mechanism in terms of chromosome units in egg production of Drosophila melanogaster.***——A cross between two inbred strains of *Drosophila melanogaster* showed an increase in egg production of almost 100 percent above the average of its parents. Genetic analysis demonstrated that significant amounts of heterosis were contributed by each chromosome pair. The total heterotic increase equaled the sum of the individual chromosome effects—that is, no interactions or combination effects could be detected. The relationship, therefore, between vigor as measured by egg production and chromosomal heterozygosis was definitely linear. The heterosis due to the individual chromosomes proved to be proportional to their "active" length as measured by band number in the salivary chromosomes and by crossover units. The physical length of the salivary and metaphase chromosomes and the percentage of visible loci showed a significant but not equally close fit.——These were the most important results of the study which was based on the following procedure. By a genetic technique involving dominant markers and inversions all possible homozygous and heterozygous combinations of the first three chromosomes from the two inbred lines were obtained forming a balanced 3×3×3 factorial design. This design permitted an efficient and accurate statistical evaluation of the factors responsible for heterosis. Environmental differences were eliminated by testing all chromosomal types at the same time in a randomized complete block experiment.

STRONG, L. C., Yale University School of Medicine, New Haven, Conn.: ***Maternal inheritance of fluorescence of Harderian glands in mice.***——Studies with ultraviolet light have disclosed the facts (1) that the Harderian glands of $C_3H$ mice have a pronounced red fluorescence, and (2) that the Harderian glands of JK mice have very little or no red fluorescence under the same conditions. In the present study, data are presented which show the following new facts: (1) the degree of fluorescence in the Harderian glands of mice of the

$F_1$ generation is intermediate between the readings for mice of the two ancestral stocks, and (2) the female parent contributes more of the characteristic of fluorescence to the $F_1$ than does the male. Thus a further parallel is drawn between the presence of protoporphyrin in the Harderian gland and susceptibility to give rise to spontaneous tumors of mammary origin.

TIMM, E. W., and E. W. LINDSTROM, Iowa State College, Ames, Iowa: *Experimental proof of mutation in virulence of the bacterial wilt pathogen of maize.*——Beginning with a single cell of *Phytomonas stewartii* with medium plus virulence, 55 mutants were isolated and stabilized. These 55 mutant strains, plus six parental strains and three controls, total 64, were tested for virulence in a triple-lattice greenhouse experiment by individual plant inoculation of a highly susceptible, sweet corn inbred line. The 55 mutant cultures exhibit highly significant differences in degree of virulence as measured by a lesion index and by a plant-weight index. Most variants emerge with a level of virulence lower than that of the parents. Three of the mutants showed a higher level of virulence, two of them being significantly higher than the parental mean. The 55 mutants differed also in colony morphology, breeding as true for this morphology as did the parental strain.

VILLEE, CLAUDE A., University of North Carolina, Chapel Hill, N. C.: *Phenogenetic studies of the aristapedia alleles of Drosophila melanogaster.*——Normally in aristapedia the entire arista is transformed into a tarsus, complete with bristles and claws, whereas in aristapedia-Bridges only the base of the arista is changed to a fleshy lobe, the rest being plumose. The expression of these mutants may be altered greatly by exposing developing larvae to cold (14.14°C) or heat (29 or 35°C) treatments for various lengths of time and during various days of larval life. Both the heat and cold treatments have their greatest effect in what corresponds to the fourth day of normal development. Cold treatments increase the expression of aristapedia-Bridges, in extreme instances approximating the aristapedia phenotype. Heat treatments decrease the expression of both aristapedia-Bridges and aristapedia. Cold treatments of aristapedia show no effect, and cold treatments of heterozygous aristapedia larvae do not alter the dominance. The leg tarsi of the two stocks react differently to cold treatments: the legs of aristapedia-Bridges flies show marked defects, being reduced in size and in number of segments, whereas aristapedia leg tarsi are not affected by the treatment. By setting up a stock combining aristaless and aristapedia-Bridges, it was found that at normal temperatures the antennal appendage acts like an arista and is reduced in size by the action of the aristaless gene; when given cold treatments, it acts like a tarsus and is no longer affected by aristaless. Cold treatments therefore render aristapedia-Bridges physiologically as well as morphologically similar to aristapedia, since aristapedia antennal tarsi are also unaffected by aristaless.

WHITING, ANNA R., University of Pennsylvania, Philadelphia, Pa.: *Hatchability and chromosome changes of Habrobracon eggs X-rayed in prophase I and*

*metaphase I.*——Dosage-hatchability curve of unfertilized eggs X-rayed in metaphase I indicates that death results from one event. (Lethal dosage 1250 r.) There is no evidence for recovery. Absence of a low dosage threshold for injury and of a high one for chromosome clumping and retardation (up to 15,000 r) eliminates chromosome stickiness as a factor. After treatment, fragments (double, terminal deletions?) are found in telophase I spindle or between metaphase II plates; occasionally smaller ones (single?) are found in one or both telophase II spindles. No bridges have been observed before cleavage. Percentages of eggs without fragments are higher than corresponding hatchability percentages. The two curves are roughly parallel. Fragments per treated nucleus vary as the 1.59 power of dosage for 250 r and 500 r. This decreases with each increase in dosage, but data are not statistically significant. These phenomena suggest that broken metaphase chromosomes, like those in spermatozoa, undergo neither restitution nor recombination while condensed.——Eggs X-rayed between synapsis and diakinesis (prophase I) have lethal dosage of about 40,000 r. Chromosomes in these stages do not react with Feulgen. Dosage-hatchability curve, almost identical in three independent experiments, depends upon unknown combinations of 1-hit and multiple-hit events, is complex and has not been analyzed. No cytological effects appear at low dosages. This removes possibility that high resistance depends upon extensive elimination of dicentrics in division I. At high dosages bridges have been seen in divisions I and II.——Cleavage of eggs treated in either stage may show bridges or may show fragments produced by two breaks in a bridge.

WOOLLEY, GEORGE W., Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine: *"Misty," a new coat color dilution in the mouse, Mus musculus.*—— An exceptionally light-colored male mouse was found in the Jackson Laboratory dilute brown strain of mice (JAX dba, *ddbbaa*). The exceptional male also possessed a white tail tip and a unilateral rectangular shaped white belly spot. Marked variations in color are not characteristic of the parental stock. The variation has proven to be distinct from other dark eye mutations causing general color dilution in the mouse. Three locations for these have been known for a number of years, the albino allels $c^e$ and $c^{ch}$, leaden *lnln* and dilution *dd*. One of the few single alphabetical symbols not now used in mice *m* (misty) was used to designate this mutant gene. *mmbbaa* and *mmBBaa* mice are more intense in color than *lnlnbbaa* and *lnlnBBaa* or *ddbbaa* and *ddBBaa* mice. *mm* allows more pigment in extremities such as the hair tip, ears and tail than either *lnln* or *dd*. The light underfur of *mm* is useful in classification. The above mutation is the first general coat color variation noted and published on in the dilute brown strain since it was originated by DR. C. C. LITTLE in 1909. The strain has been produced in large numbers during its many years of existence and used extensively in genetic and physiological studies in many countries. The particular subline in which the mutation occurred had not been crossed any wider within the strain than brother-sister for 26 generations. It has been possible to continue the inbreeding and at the same time produce a new true breeding misty-dilute, (that is, *mmddbbaa*) branch of the stock. The white tail

tip and an occasionally belly spot is a characteristic of the new branch but not of the *Mmddbbaa* segregates which are maintained in part of the new substrain.

ZAMENHOF, S., Biological Laboratory, Cold Spring Harbor, N. Y.: *Proteolytic enzymes and mutations.*——This experiment was designed to test whether proteolytic enzymes in sublethal doses are able to split "non-vital" linkages in the gene molecule and produce mutations. Sublethal doses of trypsin and pancreatin have been introduced by injections into Drosophila larvae. In addition, eggs younger than 1 hour (timed) have been treated with trypsin solution. The mortality was 75 percent due to proteolytic action. Surviving animals were tested by means of *ClB* method. The test did not show any increase in mutation rate. It seems therefore that the proteolytic enzymes are unable to produce mutational changes prior to death of the cell; however, it is also possible that due to the selective permeability of the cell membranae the enzyme cannot penetrate into living nuclei of the germ cells.

ZAMENHOF, S., and M. DEMEREC, New York, N. Y., and Carnegie Institution of Washington, Cold Spring Harbor, N. Y.: *Heavy water and mutations.*——Sublethal doses of heavy water have been introduced into Drosophila larvae by feeding and by injections. Because of the high degree of penetration, it might have been supposed that the deuterium was built into the gene molecule. Treated males were tested for lethals by the *ClB* method. Cultures were raised on standard food. The test did not show any increase in the mutation rate. It is concluded that deuterium in the gene molecule did not change the structure of the latter, or else that this structure was changed temporarily but reverted again when standard food became available.

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